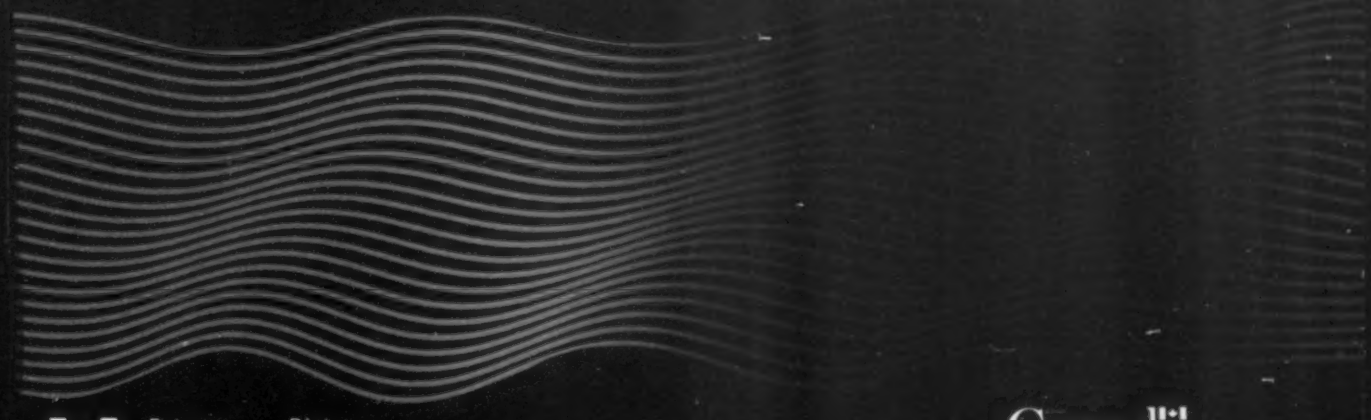
Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques



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Issues from 1934 through the current volume can be purchased on 16- or 35-mm microfilm. Photocopies of individual articles or issues can be purchased from Xerox University Microfilms, 300 North Zeeb Road, Ann Arbor, Michigan 48106, USA.

Typeset and
Printed in Canada by: The Runge Press Limited, Ottawa

Composé et
Imprimé au Canada par: The Runge Press Limited, Ottawa

Design: Ludvic Saleh & Associates, Ottawa

Conception graphique: Ludvic Saleh & Associates, Ottawa

Publié sans interruption depuis 1901, le *Journal canadien des sciences halieutiques et aquatiques* a paru sous plusieurs titres : *Contributions to Canadian Biology* 1901–1925, *Contributions to Canadian Biology and Fisheries* 1926–1934, *Journal of the Biological Board of Canada* 1934–1937 et *Journal de l'office des recherches sur les pêcheries du Canada* 1938–1979.

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Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

Volume 48, No. 11, November 1991 **Volume 48, nº 11, novembre 1991**

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Morphometric and Electrophoretic Approaches to Discrimination of Capelin (*Mallotus villosus*) Populations in the Estuary and Gulf of St. Lawrence

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Roby, D., J. D. Lambert, and J. M. Sévigny. 1991. Morphometric and electrophoretic approaches to discrimination of capelin (*Mallotus villosus*) populations in the Estuary and Gulf of St. Lawrence. Can. J. Fish. Aquat. Sci. 48: 2040–2050.

The degree of differentiation between capelin (*Mallotus villosus*) populations in the Estuary and Gulf of St. Lawrence was evaluated using conventional and truss morphometric analyses together with electrophoretic analyses of protein-coding loci. Samples of spawning fish were collected in the same year at eight sites across NAFO divisions 4R, 4S, and 4T. While the results of conventional morphometric analyses were not conclusive, truss and electrophoretic analyses differentiated the sampled sites along an east–west axis. Two samples (one from the Southern Gulf and the other from the Northeastern Gulf) did not follow this trend. Processes are suggested to explain this pattern. Variations in allelic frequencies among age-classes of fish have been observed in some samples and may be attributed to selective environmental pressures. This could explain the apparent population differentiation observed, although the distribution of the alleles revealed a high gene flow.

Le degré de différenciation des populations de capelan (*Mallotus villosus*) de l'estuaire et du golfe du Saint-Laurent a été évalué par des analyses morphométriques conventionnelles et en réseau et par des analyses électrophorétiques de protéines enzymatiques. Huit échantillons de poissons en état de frai ont été prélevés dans les régions 4R, 4S et 4T de l'OPANO. Les résultats de l'analyse morphométrique conventionnelle furent peu concluants. Les analyses morphométriques en réseau et les analyses électrophorétiques ont mis en évidence une différenciation des sites échantillonnés selon un axe est–ouest. Toutefois, deux échantillons, l'un du sud du golfe et l'autre du nord-est du golfe n'ont pas suivi cette tendance. Des processus ont été suggérés afin d'expliquer la particularité de ces échantillons. Des variations de fréquence de certains allèles entre les classes d'âge de poissons récoltés à certains sites ont été observées et pourraient être attribuées à des pressions de sélection environnementale. Ceci expliquerait la différentiation apparente des populations malgré que la structure des populations ait été caractérisée par un flux génique élevé.

Received October 22, 1990
Accepted May 9, 1991
(JA768)

Reçu le 22 octobre 1990
Accepté le 9 mai 1991

The ecological role of capelin (*Mallotus villosus*) in the marine food web as food for predatory species (Jangaard 1974; Baily et al. 1977; Akenhead et al. 1982) and its economic importance as a commercially exploited species justify the need for an understanding of the mechanisms underlying its population dynamics. A sustainable exploitation of this resource requires knowledge of population genetic structure in order to define appropriate management units or stocks and to avoid genetic changes or losses caused by long-term selection due to fishing pressure (Law and Grey 1988; Rowell et al. 1989).

The population structure of capelin in the Estuary and Gulf of St. Lawrence is not well defined. This resource, however, is presently managed as three separate stocks, NAFO divisions 4R, 4S, and 4T. In the Northwest Atlantic, five stocks have been identified on the basis of seasonal distribution patterns (Campbell and Winters 1973), meristics (Sharp et al. 1978; Carscadden and Misra 1980), and morphometrics (Sharp et al. 1978; Misra and Carscadden 1987). Among these presumed stocks, some authors have suggested the possible existence of complexes of stocks in the Gulf of St. Lawrence (Sharp et al. 1978; Fortier et al. 1987). Within the Estuary and Gulf of St. Lawrence, Parent and Brunel (1976) have delineated four areas based on different spawning periods possibly determined by different production cycles. Results of a multivariate morphometric analysis have indicated a strong separation between four groups from the Gulf (Sharp et al. 1978). Using the same discriminant characters on samples from similar areas, Lambert and Bernier (1989) did not obtain such a clear separation. No study of the genetic structure of capelin populations in the Northwest Atlantic has yet been published, except for an analysis of esterase polymorphism (Payne 1975, 1976).

The objective of the present study is to describe the population structure of capelin in the Estuary and Gulf of St. Lawrence. The concomitant use of two different approaches, a genetic analysis and a morphometric description of each individual, should improve the understanding of the population structure and result in an estimate of the level of exchange or migration between them.

Material and Methods

Sampling

Eight sites across the Estuary and Gulf of St. Lawrence in each of NAFO divisions 4R, 4S, and 4T were sampled between

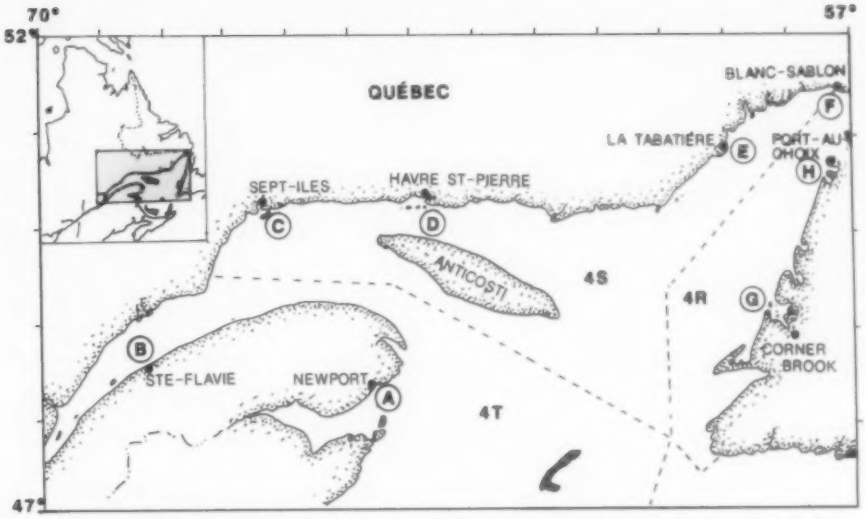

FIG. 1. Geographic location of sampling sites within the Estuary and Gulf of St. Lawrence. Each site is denoted by a code letter and NAFO divisions are indicated.

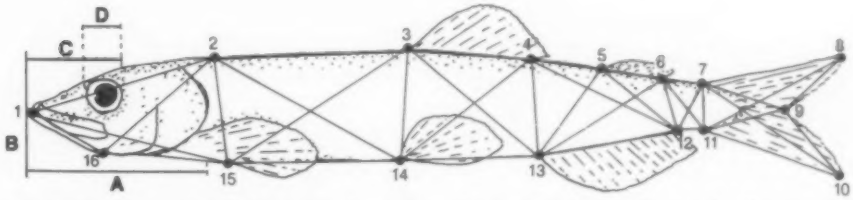

FIG. 2. Characters measured for truss and conventional morphometric analysis. Landmarks are numbered and morphometric distances are illustrated by lines. Characters measured for conventional analysis: A = head length; B = body depth; C = snout length; D = eye diameter.

May and July 1989 (Fig. 1). Fifty females and fifty males in spawning condition were collected at each location. Conventional and truss morphometric measurements were obtained by laying the fish on water-resistant paper and inserting pins in the paper at selected landmarks (Fig. 2). These data were digitized using a digitizing board and rounded to 0.1 mm. Muscle and liver samples from each specimen were stored at −80°C for electrophoretic analysis.

Numerical Analysis and Characters Measured

Conventional data

The conventional morphometric characters used were head and snout length, eye diameter, and body depth. These characters have been shown to be significant discriminators between capelin populations from the Gulf of St. Lawrence (Sharp et al. 1978). Assuming that morphometric characters are linearly related to standard length, a linear geometric mean regression (Ricker 1973) was used to correct for length (Meng and Stocker 1984). Logarithmic transformation of data was unnecessary due to the small variation between length-classes. Males and females were analysed separately throughout the statistical analysis because of their sexual dimorphism (Jangaard 1974; Misra and Carscadden 1987). Mahalanobis distance was calculated between the groups and converted into overlapping percentages (Mais 1972) using Royce's (1964) concept. Differences among the samples were visualized by discriminant function analysis to evaluate the degree of morphometric separation. This discriminant function was used to classify individuals into groups employing the jacknife technique.

Truss data

Bookstein's (1978) geometric approach was used for the truss morphometric analysis. A modified principal component analysis was used to remove the size effect (Somers 1986). This method extracts a first isometric size factor, allowing the subsequent components to be interpreted as summarizing shape variation independent of size and random variation among the sampled individuals. The analysis was carried out using the 1989 edition of the program "SIZE" (Somers 1986).

The variables showing maximum variability in the allometric components were selected as a reduced data set for the discriminant analysis as well as for the calculation of Mahalanobis distance and of the overlapping percentages between the sampled groups (Royce 1964; Mais 1972). A jacknife classification of individual fish into groups was effected using the discriminant function.

Electrophoretic Analysis

Samples (100 mg) of tissue were homogenized in extraction solution (0.2 mL for muscle and 0.4 mL for liver) containing

TABLE 1. Description of the observed enzymatic systems. Number of observed alleles corresponds to those resolved in the samples (n.a. = not analysed). M = muscle; L = liver. No polymorphism was observed at the loci coding for the following enzymes: G6PDH, FH, LDH, FBALD, TPI, CK, AK, ADA, and XDH in the muscle; ADH, LAP, ACP, PFK, XDH, and AO in the liver.

Enzyme	EC. No.	Observed loci	Tissue	No. of observed alleles
Esterases (EST)	3.1.1.-	1	L	5
		2	M, L	9
		3	M	n.a.
		4	M	n.a.
Glucose-6-phosphate isomerase (GPI)	5.3.1.9	1	M	4
		2	M	8
L-Iditol dehydrogenase (IDDH)	1.1.1.14	1	L	5
Malate dehydrogenase (MDH)	1.1.1.37	1	M	3
		2	M	3
Malic enzyme ($NADP^+$) (MEP)	1.1.1.40	1	L, M	5
Mannose-6-phosphate isomerase (MPI)	5.3.1.8	1	L, M	4
Phosphoglucomutase (PGM)	5.4.2.2	1	M	6

0.01 M Tris–HCl (pH 8.0), 30% sucrose, 0.005 M dithiothreitol (DTT), and 0.5% polyvinylpolypyrrolidone (PVP). Phenylmethylsulfonyl fluorure (PMSF) (0.001 M) was added to the solution for liver extracts. Homogenates were centrifuged at 2400*g* for 20 min at 4°C.

Thirty-three enzymes were assayed on either discontinuous polyacrylamide slab gels (Ornstein 1964) or cellulose acetate gels (Hebert and Beaton 1989). The staining of enzymes followed the methods of Harris and Hopkinson (1976). The loci analysed for all of the fish are listed in Table 1. The gene nomenclature for protein-coding loci follows the recommendations of Shaklee et al. (1990).

Statistical analysis

Statistical analysis was carried out in part with the BIOSYS-1 computer programme (Swofford and Selander 1989). Conformance to Hardy–Weinberg proportions was tested using a *G*-test for goodness-of-fit. Allelic frequency differences among populations and among age-classes within each population were tested for significance with the heterogeneity *G*-test and allele or age-classes were pooled whenever expected frequencies were too small (Sokal and Rohlf 1981). Absolute differentiation between populations was estimated using Nei's (1978) unbiased and Cavalli-Sforza and Edwards' (1967) genetic distances. A matrix of Cavalli-Sforza and Edwards distances between each pair of populations was calculated for the pooled loci and for each individual locus. A principal coordinate analysis, on which a minimum spanning tree was superimposed, was computed from the pooled matrix using NTSYS, version 1.40 (Rohlf 1988).

The relative differentiation among populations versus overall variation was evaluated using Wright's (1965) fixation index. The existence of subgroups was verified using a hierarchical analysis of population differentiation (Wright 1978). The analysis was performed on the subgroups specified based on NAFO divisions and on subgroups based on their genetic relatedness as revealed by the ordination of the genetic distances matrix.

Gene flow and the number of migrants were estimated from Wright's (1969) fixation index according to the formula

$$F_{ST} = 1/(1 + 4N_e m)$$

where m = migration rate and N_e = effective number of individuals. Gene flow was also qualitatively evaluated according to Slatkin's (1981) method of rare alleles. This method, based on the frequency and incidence of individual alleles among populations, calculates the conditional average frequency, $\bar{p}_i$, corresponding to the unweighted mean of all alleles occurring in i of the d sites.

Results

Conventional Morphometric Analysis

Overlapping ratios based on Mahalanobis distances (Table 2) showed no distinct separation among samples. A posteriori classification percentages were low (Table 3) and varied between 8% (Corner Brook) and 70% (Sept-Îles), averaging around 40%. These percentages are low compared with those of Misra and Carscadden (1987) and Sharp et al. (1978) who claimed up to 100% correct classification.

In the scatter diagram of individual scores (Fig. 3), samples from La Tabatière (E) and Ste. Flavie (B) separated from Sept-Îles (C) on the first canonical axis, while these three samples (for both males and females) separated from Havre St. Pierre (D) on the second axis.

Truss Morphometric Analysis

The first eigenvalue summarizing size covariation among the variables accounted for 30 and 49% of the variance in males and females, respectively. The two subsequent eigenvalues, interpreted in terms of shape, accounted for 14 and 7% of the total variance in males and for 9 and 6% in females. The descriptors contributing most to these two components were the dimensions of the caudal peduncle, abdomen, adipose fin, and caudal fin for both sexes. These variables were selected for further analyses.

The projection of individuals in the reduced space of the second and third components (Fig. 4) illustrated a general west (samples A and C) versus east (samples F, G, and H) grouping tendency of the stations. The La Tabatière sample (E) was distinct from the Newport sample (A) along the second axis for males and along the third axis for females. These axes were determined mainly by the longitudinal component of the body shape, the distance between the insertions of the pectoral and pelvic fins for males and that between the insertions of the pelvic and anal fins for females.

Overlapping ratios between the groups sampled were lower than those obtained with the analysis of conventional characters (Table 2). For males, the La Tabatière sample is clearly distinct from the other sites with the exception of Ste. Flavie. This is consistent with the distribution of individuals on the scatter diagram (Fig. 4). For females, the La Tabatière and Newport samples are differentiated only from the eastern samples. If one considered two geographical regions, one in the eastern and the other in the western sampling zone, excluding La Tabatière sample, intraregional overlapping appeared to be more important than interregional overlapping.

A posteriori classification of individuals (based on selected variables) reclassified them in the region from which they originated, if not in their respective group (Table 4).

The result of the morphometric study suggested a possible differentiation along an east–west axis in the Gulf of St. Law-

TABLE 2. Percent overlap comparisons based on Mahalanobis distances of conventional (above diagonal) and truss (below diagonal) male and female morphometric variables. Letters refer to groups as in Fig. 1.

Group	A	B	C	D	E	F	G	H
				Males				
A	—	75.66	23.02	41.22	61.00	47.76	56.20	65.28
B	6.28	—	14.16	29.84	61.00	34.72	41.80	47.76
C	45.32	14.16	—	27.14	20.06	23.02	23.80	28.92
D	58.92	17.38	72.64	—	22.62	47.16	35.24	28.92
E	0.24	19.70	0.76	0.88	—	28.92	35.76	58.92
F	23.40	9.70	29.38	18.68	1.18	—	70.80	72.64
G	42.38	10.30	53.52	53.52	0.72	60.30	—	63.12
H	24.20	10.30	31.75	22.24	1.04	72.64	61.70	—
				Females				
A	—	52.86	61.70	37.88	19.02	30.78	42.78	55.52
B	16.76	—	34.22	59.92	33.20	52.86	61.70	54.18
C	39.54	42.38	—	27.14	17.70	24.20	34.72	37.34
D	18.68	71.88	49.02	—	30.78	42.38	77.94	47.76
E	12.86	22.24	25.42	16.46	—	69.66	42.38	33.20
F	7.50	34.72	14.98	39.54	3.58	—	54.18	50.28
G	7.02	30.30	12.60	34.22	2.78	80.26	—	57.54
H	2.64	12.36	5.12	16.16	0.86	51.56	48.40	—

TABLE 3. Percent correct a posteriori classification to groups based on morphometric classification functions of conventional variables. Letters refer to groups as in Fig. 1.

	Number of individuals								Percent correctly
Group	A	B	C	D	E	F	G	H	classified
					Males				
A	15	11	5	1	10	0	8	0	30
B	5	25	1	4	8	1	4	2	50
C	0	1	35	3	2	3	3	3	70
D	3	0	5	30	1	5	1	5	60
E	3	9	1	2	31	0	3	1	62
F	4	4	5	4	3	16	11	3	32
G	6	5	3	2	2	10	20	2	40
H	6	6	5	11	6	6	6	4	8
					Females				
A	21	4	12	2	1	3	0	7	42
B	3	16	3	5	2	10	5	6	32
C	10	2	29	2	0	0	1	6	58
D	1	7	4	18	5	3	8	4	36
E	0	2	1	3	30	8	3	3	60
F	1	8	1	3	14	13	3	7	26
G	2	3	6	18	3	6	5	7	10
H	2	8	7	2	4	3	4	20	40

rence. Certain regions such as Newport (sample A) and La Tabatière (sample E), however, stood out from these groups.

Electrophoretic Analysis

Fifteen loci were satisfactorily resolved in the muscle and 10 in the liver. Genetic variation was observed at 10 of these (Table 1). Allelic frequencies for the polymorphic loci, analysed sample sizes, and observed and expected heterozygosity values are given in Table 5 for each sample.

The interpretation of the banding patterns followed that of Payne (1975, 1976), Dushchenko (1984), and Sorensen and Simonsen (1988) for muscle and liver esterases. Discrepancies occurred in the number of observed loci for some enzyme systems (MDH, Sorensen and Simonsen 1988; MDH and PGM, Mork and Friis-Sorensen 1983) and in the number of observed alleles and their frequencies (MPI, PGM, MEP, and GPI, Sorensen and Simonsen 1988; MEP, GPI, and PGM, Mork and Friis-Sorensen 1983).

The comparison of observed genotypic frequencies with expected Hardy–Weinberg proportions indicated a significant deviation ($p < 0.005$) at some loci (*IDDH**, Sept-Îles; *GPI-1** and *GPI-2**, Havre St. Pierre; *PGM**, La Tabatière; *EST-2**, Ste. Flavie; and *EST-1**, Ste. Flavie, Blanc-Sablon, Corner Brook, and Newport). An excess of homozygotes was found in most samples for the two esterase loci but it was not always significant. The results of this test confirmed the genetic interpretation of banding patterns for all of the enzymatic systems except the liver esterases. The significant disequilibrium at this locus in four of the eight samples could be attributed to selection and/or absence of panmixia (Fairbairn 1981). Doubtful or unusual phenotypes were rerun along with standards to ascertain the classification of alleles.

Within-sample variation based on sex and age-class was analysed for each sample. For many loci, the relative frequency of individual alleles or pairs of alternative alleles followed a gradual change. In many samples, the direction of these allelic frequency changes was opposite in the east versus the west. Among these, in the Port-au-Choix and Corner Brook samples, the frequency of *MEP*100* increased, while that of *MEP*120* decreased. In the Ste. Flavie and Sept-Îles samples, the frequency of *MEP*100* decreased, while *MEP*120* increased, resulting in an increase of heterozygosity with age.

In samples from Havre St. Pierre, La Tabatière, Blanc-Sablon, Port-au-Choix, and Corner Brook, the frequency of *EST-1*100* increased while the frequency of *EST-1*50*

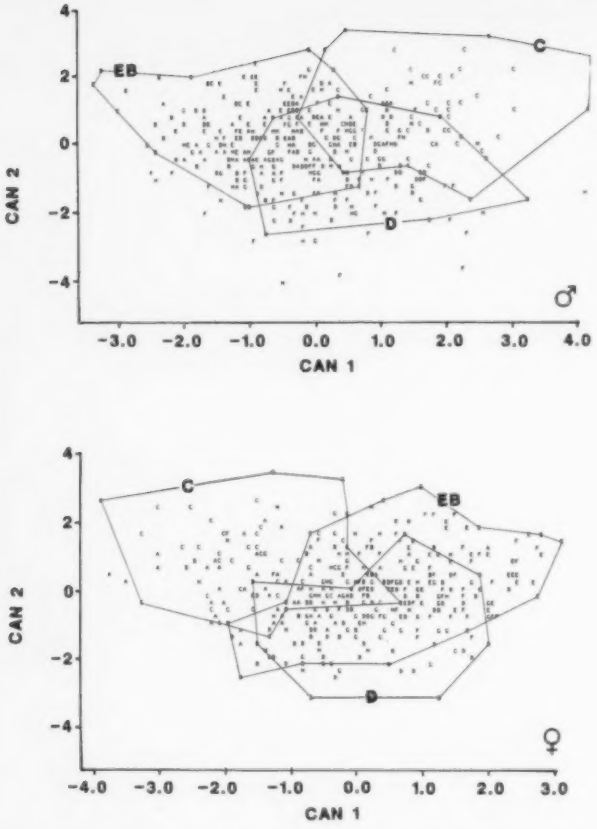

FIG. 3. Scatter diagram of discriminant function scores along the first two canonical axes for male and female capelin using conventional morphometric characters. Letters refer to sample locations as in Fig. 1.

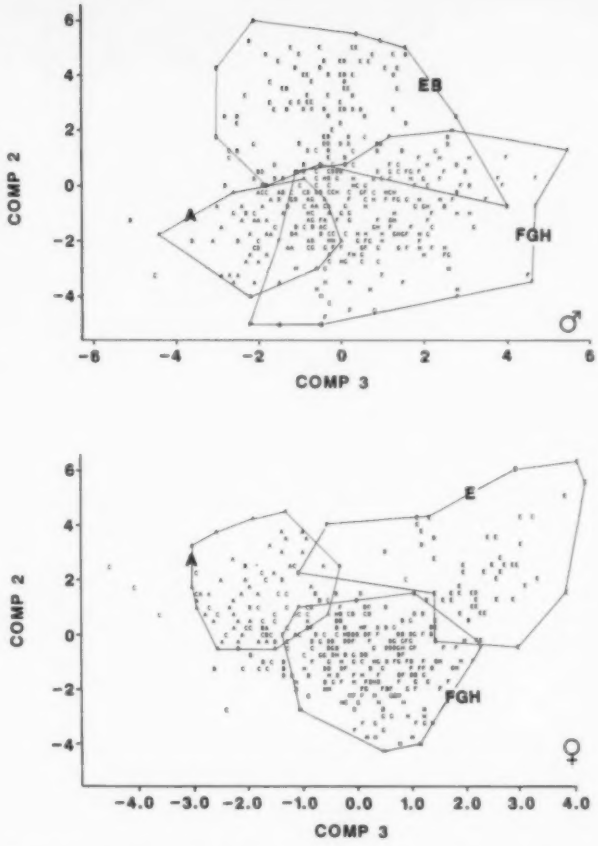

FIG. 4. Scatter diagram of principal component scores along the second and third allometric components for male and female capelin using truss morphometric characters. Letters refer to sample locations as in Fig. 1.

decreased. With the exception of Havre St. Pierre, these changes caused a reduction of heterozygosity in the samples.

For the locus *EST-2**, the frequency of the second most common allele **94* increased with age in all samples, while the frequency of the most common allele **100* increased in Havre St. Pierre, Port-au-Choix, and Corner Brook while diminishing in Ste. Flavie.

These allelic frequency changes were not statistically significant which could be attributable to the small effective sample size of each age-class. Their east–west consistency ought nevertheless to be considered.

Heterogeneity *G*-test results at the individual loci *MEP**, *PGM**, and *IDDH** as well as for the pooled loci (Table 6) rejected the null hypothesis that the eight samples were derived from a single population.

The weighted means of allelic F_{ST} values for each locus (Table 6) were small but always above zero. Among the loci showing the highest values of F_{ST}, *GPI-1** was the only one lacking significant heterogeneity among samples from the G_H test. The mean value of F_{ST} over all loci was 0.005. Genetic heterogeneity was thus shown among the sampled populations, and a certain portion of this variance, albeit small, was attributed to differences between the samples.

Nei's unbiased genetic distances ranged from 0.000 to 0.002. A Cavalli-Sforza and Edwards genetic distance matrix calculated over loci for all pairwise combinations of samples (Table 7) was represented in the ordination presented in Fig. 5. The first three axes of this principal coordinate analysis summarized a total of 51% of the matrix information. This ordination, on which a minimum spanning tree was reported, emphasized the central position of Havre St. Pierre (D) relative to the overall distribution.

Although each coordinate could not be individually interpreted, a clear distribution of samples along the second axis corresponded to their geographic distribution along an east–west cline. La Tabatière (E) and Port-au-Choix (H), however, stood out from the Corner Brook (G) and Blanc-Sablon (F) pair of samples. This figure also illustrated the proximity of the Ste. Flavie (B) and Sept-Îles (C) samples from which Newport (A) was isolated.

The distance matrices calculated for each locus showed that Newport was most differentiated from the other samples at loci *MEP** and *GPI-2** for which two private alleles were expressed (Table 5). The Port-au-Choix sample was most differentiated at locus *EST-2** and La Tabatière at loci *EST-2** and *MDH-1**. The presence of a high percentage of 5-yr-old individuals in the La Tabatière sample (Table 8) contributed to its genetic differentiation. In effect, the 5-yr-olds possessed an allele,

TABLE 4. Percent correct a posteriori classification to groups based on morphometric classification functions of truss variables. Letters refer to groups as in Fig. 1.

Group	Number of individuals								Percent correctly classified
	A	B	C	D	E	F	G	H	
Males									
A	31	0	9	3	0	0	6	1	62
B	0	31	7	1	11	0	0	0	62
C	5	0	22	18	0	0	4	1	44
D	5	2	7	36	0	0	0	0	72
E	0	13	0	0	37	0	0	0	74
F	1	2	8	0	0	19	5	15	38
G	3	0	8	8	0	13	11	6	26
H	2	1	4	1	0	13	5	24	48
Females									
A	34	0	9	1	6	0	0	0	68
B	1	21	8	6	5	6	3	0	42
C	5	4	31	7	3	0	0	0	62
D	0	12	9	17	2	5	5	0	34
E	0	6	1	0	43	0	0	0	86
F	0	4	3	4	0	14	12	13	28
G	0	5	0	2	0	8	24	11	48
H	0	2	1	0	0	3	18	26	52

*MDH-2*120*, not found in younger individuals in any sample. Other alleles such as *EST-2*83* and **115*, *GPI-2*85*, and *PGM*87* were expressed only, or at a much higher frequency, in these 5-yr-old individuals.

The results of a first Wright hierarchical analysis performed on samples grouped according to their respective NAFO dividions, 4R, 4S, and 4T, were compared with the results of the same analysis where samples were clustered according to the previously calculated genetic distances, i.e. grouping Ste. Flavie, Sept-Îles and Havre St-Pierre, and Blanc-Sablon, Corner Brook and Port-au-Choix, with Newport and La Tabatière being kept separate (Table 9). The analysis performed on the NAFO divisions inflated the variability within an area compared with the overall variance resulting in null or negative values for the variance between divisions. Results of the genetic grouping apportioned more variability between areas for most of the loci and for the mean calculated over loci. This was not only due to the fact that Newport and La Tabatière samples were kept separate in this analysis. They contributed to, but did not account for, the major part of the total variance at the loci with higher F_{ST} values.

Gene Flow Estimation

Values of N_em calculated at each locus (Table 6) were high and relatively similar except for *EST-1**. Assuming that the differences between loci were random sampling differences, i.e. not due to selection, an overall value of 41 was estimated from F_{ST} mean value over loci. However, a disequilibrium of the genotypic proportions was detected at this locus and at the *EST-2** locus for most samples. The possibility that this disequilibrium might have been the result of selective pressure could not be ruled out. Mean F_{ST} was therefore recalculated without taking into account these two loci and the resulting N_em reestimated at 31 migrants per generation.

The qualitative estimation (Fig. 6) also indicated a high level of apparent gene flow according to Slatkin's (1981) simulations.

Discussion

Our results revealed a nonrandom morphometric differentiation and significant genetic heterogeneity among capelin samples from the Estuary and Gulf of St. Lawrence. Results of both approaches were congruent and suggested the existence of two regions: a western region comprising Ste. Flavie, Sept-Îles, and Havre St. Pierre, with Newport being different from these sites, and an eastern region comprising Blanc-Sablon, Corner Brook, and Port-au-Choix, with La Tabatière being different from those sites. This population structure of capelin in the Gulf of St. Lawrence was drawn mainly from the results of the calculation of percent overlap, principal component analysis of truss characters, and from the ordination of genetic distances. A comparison of the results of hierarchical analyses of genetic variability indicated that NAFO areas corresponded much less to the genetic relatedness of the sampled populations than the suggested subdivisions.

A better discrimination between these groups of samples was obtained with the truss morphometric approach than with the conventional approach. Truss variables provided a much more detailed description of capelin shape than the significant characters found by Sharp et al. (1978), although these authors had observed lower overlapping ratios for populations from the Gulf of St. Lawrence. The validity of those characters had been questioned by Lambert and Bernier (1989), since their significance varied between years and between sexes.

In the Northwestern Atlantic, Payne (1975, 1976) analysed esterase polymorphism of capelin populations from West Greenland and eastern Newfoundland. Further east, capelin populations from the Barents Sea (Mork and Friis-Sorensen 1983) and the west coast of Greenland (Sorensen and Simonsen 1988) have been studied. Mork and Friis-Sorensen and Simonsen (1983) observed 1, 5, and 1 alleles for *MEP**, *GPI**, and *PGM** loci, respectively, separated by isoelectric focusing, while 5, 8, and 6 alleles were observed in the present study. Discrepancies with our results may be attributed to differences in analytical methods. The Barents Sea capelin may also be characterized by a different genetic structure. Sorensen and Simonsen (1988) analysed their samples using starch gel electrophoresis. Major differences existed with (1) *MPI** where we observed one major allele, while they found two alleles segregating almost equally within the population, and with (2) *MEP** where we found five alleles with frequencies of the two most frequent ones averaging 0.72 and 0.27, while they observed one major (0.98) and one rare allele (0.02).

The east–west geographic differentiation of capelin populations within the Gulf of St. Lawrence may have resulted from a simple process of isolation by distance where gene exchange occurs preferentially between neighbouring populations. That some samples stood out from this pattern remains to be explained. The Port-au-Choix sample differentiation was genetic but not morphometric and no obvious explanation could be derived from our data.

Within the eastern region, age structure (Table 8) and length–frequency analysis of the La Tabatière sample indicated the presence of older and larger individuals compared with other sites (J. D. Lambert, unpubl. data). According to local fisheries observers and fishermen, two distinct spawning waves occur along the lower North Shore of Quebec: the first wave

TABLE 5. Allelic frequencies, observed (H_o), and expected (H_e) heterozygosities at polymorphic loci (N = sample size).

Locus/ allele	Sampled site							
	Ste. Flavie	Sept-Îles	Havre St. Pierre	Blanc-Sablon	Corner Brook	Port-au-Choix	La Tabatière	Newport
MEP								
N	100	100	100	100	100	100	100	100
**100*	0.680	0.715	0.690	0.770	0.765	0.660	0.695	0.775
**120*	0.315	0.275	0.295	0.215	0.235	0.325	0.300	0.205
**115*	0.000	0.010	0.010	0.010	0.000	0.015	0.005	0.000
**85*	0.005	0.000	0.000	0.005	0.000	0.000	0.000	0.015
**130*	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.005
H_o	0.420	0.410	0.400	0.360	0.390	0.440	0.490	0.410
H_e	0.438	0.413	0.437	0.361	0.360	0.459	0.427	0.357
MDH-1								
N	100	100	100	100	100	100	100	100
**100*	0.995	0.995	1.000	1.000	1.000	1.000	0.990	1.000
**130*	0.005	0.005	0.000	0.000	0.000	0.000	0.000	0.000
**50*	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000
H_o	0.010	0.010	0.000	0.000	0.000	0.000	0.020	0.000
H_e	0.010	0.010	0.000	0.000	0.000	0.000	0.020	0.000
MDH-2								
N	100	100	100	100	100	100	100	100
**100*	0.985	0.995	0.995	0.965	0.965	0.985	0.985	0.990
**68*	0.015	0.005	0.005	0.030	0.035	0.005	0.010	0.010
**120*	0.000	0.000	0.000	0.005	0.000	0.010	0.005	0.000
H_o	0.030	0.010	0.010	0.070	0.070	0.030	0.030	0.020
H_e	0.030	0.010	0.010	0.068	0.068	0.030	0.030	0.020
GPI-1								
N	100	100	100	100	100	100	100	100
**100*	0.975	0.945	0.985	0.985	0.990	0.980	0.990	0.990
**87*	0.020	0.045	0.005	0.015	0.010	0.005	0.005	0.010
**120*	0.005	0.010	0.010	0.000	0.000	0.015	0.000	0.000
**40*	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000
H_o	0.050	0.110	0.020	0.030	0.020	0.040	0.020	0.020
H_e	0.049	0.105	0.030	0.030	0.020	0.039	0.020	0.020
GPI-2								
N	100	100	100	100	100	100	100	100
**100*	0.985	0.965	0.975	0.950	0.965	0.980	0.975	0.945
**90*	0.010	0.025	0.010	0.025	0.030	0.015	0.015	0.015
**110*	0.005	0.010	0.005	0.010	0.000	0.000	0.000	0.010
**85*	0.000	0.000	0.010	0.010	0.005	0.000	0.005	0.010
**94*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.015
**117*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005
**87*	0.000	0.000	0.000	0.005	0.000	0.005	0.000	0.000
**nul*	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000
H_o	0.030	0.070	0.030	0.100	0.070	0.040	0.050	0.110
H_e	0.030	0.068	0.049	0.097	0.068	0.039	0.049	0.106
PGM								
N	100	100	100	100	100	100	100	100
**100*	0.870	0.905	0.895	0.850	0.895	0.885	0.835	0.900
**92*	0.085	0.055	0.065	0.125	0.045	0.065	0.110	0.050
**107*	0.025	0.030	0.020	0.005	0.010	0.030	0.045	0.040
**112*	0.015	0.005	0.005	0.010	0.020	0.005	0.005	0.005
**96*	0.005	0.000	0.005	0.005	0.005	0.010	0.000	0.000
**87*	0.000	0.005	0.010	0.005	0.025	0.005	0.005	0.005
H_o	0.250	0.180	0.180	0.250	0.180	0.210	0.250	0.200
H_e	0.235	0.177	0.194	0.262	0.196	0.212	0.289	0.186
MPI								
N	98	100	100	100	100	100	99	99
**100*	0.974	0.970	0.965	0.945	0.920	0.975	0.929	0.944
**110*	0.005	0.010	0.015	0.020	0.060	0.010	0.030	0.020
**90*	0.010	0.020	0.020	0.035	0.020	0.015	0.035	0.035
**80*	0.010	0.000	0.000	0.000	0.000	0.000	0.005	0.000
H_o	0.051	0.060	0.070	0.110	0.160	0.050	0.141	0.111
H_e	0.050	0.059	0.068	0.105	0.150	0.049	0.134	0.106

TABLE 5. (*Concluded*)

Locus/ allele	Sampled site							
	Ste. Flavie	Sept-Îles	Havre St. Pierre	Blanc-Sablon	Corner Brook	Port-au-Choix	La Tabatière	Newport
IDDH								
N	98	100	97	100	100	100	100	99
**100*	0.969	0.955	0.995	0.935	0.970	0.945	0.985	0.990
**90*	0.005	0.040	0.000	0.050	0.020	0.035	0.010	0.005
**110*	0.015	0.005	0.005	0.005	0.005	0.015	0.000	0.005
**80*	0.005	0.000	0.000	0.010	0.005	0.005	0.005	0.000
**120*	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H_o	0.061	0.050	0.010	0.110	0.060	0.090	0.030	0.020
H_e	0.060	0.086	0.010	0.123	0.059	0.106	0.030	0.020
EST-1								
N	98	100	97	100	100	100	100	97
**100*	0.663	0.685	0.644	0.665	0.670	0.680	0.636	0.624
**50*	0.301	0.280	0.309	0.300	0.265	0.280	0.316	0.325
**25*	0.026	0.030	0.026	0.015	0.015	0.020	0.010	0.021
**175*	0.005	0.005	0.021	0.020	0.050	0.015	0.031	0.031
**150*	0.005	0.000	0.000	0.000	0.000	0.005	0.000	0.000
H_o	0.378	0.400	0.443	0.340	0.450	0.410	0.439	0.361
H_e	0.469	0.451	0.488	0.467	0.478	0.459	0.486	0.504
EST-2								
N	100	100	100	100	100	100	100	100
**77*	0.010	0.005	0.005	0.000	0.010	0.000	0.000	0.000
**83*	0.015	0.010	0.045	0.040	0.030	0.030	0.005	0.035
**89*	0.100	0.115	0.105	0.140	0.100	0.120	0.080	0.070
**94*	0.210	0.210	0.180	0.175	0.205	0.260	0.200	0.220
**100*	0.290	0.270	0.245	0.240	0.270	0.250	0.290	0.350
**105*	0.155	0.160	0.195	0.170	0.200	0.170	0.230	0.150
**110*	0.125	0.150	0.120	0.135	0.100	0.070	0.105	0.110
**115*	0.065	0.055	0.080	0.065	0.070	0.090	0.070	0.050
**120*	0.030	0.025	0.025	0.035	0.015	0.010	0.020	0.015
H_o	0.660	0.800	0.730	0.830	0.760	0.790	0.690	0.700
H_e	0.817	0.818	0.835	0.838	0.819	0.813	0.800	0.786

TABLE 6. F_{ST} indices calculated for each locus and estimated values of N_em, number of migrants per generation. Significance of sample differentiation ($*p < 0.05$; $***p < 0.005$) was tested with a heterogeneity *G*-test.

Locus	F_{ST} (locus)	N_em
MEP	0.009*	28
MDH-1	0.006	41
MDH-2	0.008	31
GPI-1	0.010	25
GPI-2	0.005	50
PGM	0.007*	35
MPI	0.008	31
IDDH	0.012***	21
EST-1	0.002	125
EST-2	0.005	50
Mean over loci	0.006***	41
Mean over loci excluding esterases	0.008***	31

being composed of small whitish individuals and the second of older and larger individuals commonly called "blue back." Fishermen preferentially select the "blue back" capelin. The La Tabatière sample might have been composed of individuals from this second wave. Although different capelin spawning waves have been reported in the Estuary and Gulf of St. Lawrence (Parent and Brunel 1976; Lambert et al. 1982; Fortier et al. 1987), there is no published evidence of morphological differences between such waves.

In the western region, the similarity of the Ste. Flavie and Sept-Îles samples is probably explained by the downstream larval drift from the Estuary into the Anticosti cyclonic gyre (Jacquaz et al. 1977). Although 0-group larvae do not seem to concentrate there, the Anticosti gyre appears to be a nursery ground for juvenile capelin (de Lafontaine et al. 1981) and constitutes a possible retention zone. This may account for the existence of a single population according to the hypothesis of Iles and Sinclair (1982).

The age structure of the Newport sample (Table 8) with its prevalence of 2-yr-old individuals is probably an artefact of sampling rather than a reflection of distinct populations, since this sample was taken at the end of the spawning season. The age structure does not, however, explain the distinction of this sample from the others within the western region. Differentiation may arise because larvae and juveniles from Newport occur in a possible retention zone (de Lafontaine et al. 1984) created by the anticyclonic gyre within the Baie-des-Chaleurs (Legendre and Watt 1970) distinct from the Anticosti gyre.

Results of the genetic approach indicated a weak differentiation among samples. This is a consequence of extensive gene flow which is expected in a restricted sampling area such as the Estuary and Gulf of St. Lawrence and for a pelagic marine fish species. The estimated number of migrants is high (31) when compared with Slatkin's (1981) values calculated for organisms belonging to different taxa but is of the same order of magnitude

TABLE 7. Genetic distance matrix (Cavalli-Sforza and Edwards 1967) between samples calculated over all loci.

	Population	B	C	D	F	G	H	E	A
B	Ste. Flavie	—							
C	Sept-Îles	0.063	—						
D	Havre St. Pierre	0.069	0.070	—					
F	Blanc-Sablon	0.082	0.075	0.078	—				
G	Corner Brook	0.085	0.082	0.071	0.070	—			
H	Port-au-Choix	0.073	0.070	0.069	0.076	0.081	—		
E	La Tabatière	0.081	0.081	0.073	0.079	0.075	0.078	—	
A	Newport	0.081	0.081	0.069	0.082	0.076	0.091	0.079	—

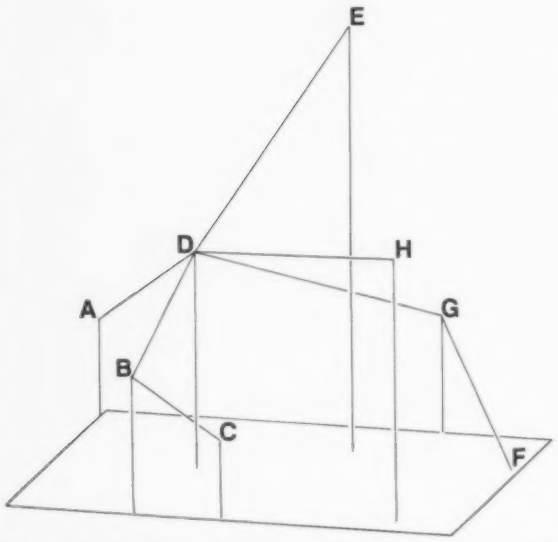

FIG. 5. Principal coordinate analysis and minimum spanning tree based on Cavalli-Sforza and Edwards' (1967) genetic distance. Letters refer to sample locations as in Fig. 1.

TABLE 8. Number of capelin of four age-classes from the sampled sites.

	Age			
Site	2	3	4	5
Newport	39	59	2	
Ste. Flavie	20	70	10	
Sept-Îles	23	69	8	
Havre St. Pierre	18	82		
La Tabatière	1	43	35	21
Blanc-Sablon	1	80	16	3
Corner Brook	9	87	4	
Port-au-choix	15	83	2	
Total	126	573	77	24

as those calculated for other marine fish species (Winans 1980; King 1983; Grant 1984; Grant and Utter 1984; King et al. 1987; Yoshiyama and Sassaman 1987; Fevolden and Haug 1988). An average estimate of the number of migrants calculated from data presented by these authors is 30. The estimated number of migrants among capelin populations in the Estuary and Gulf of St. Lawrence is comparable with Waples (1987) value of 10 estimated for high dispersal capability species in marine shore fish.

An apparent population differentiation as described in this study may be observed despite extensive gene flow if relatively strong differential selective pressures are exerted on different populations. In the assessment of population structure, a debate persists about the relative importance of evolutionary factors such as migration and selection. Selective neutrality at electrophoretic loci is a basic assumption when using allele frequency data in the analysis of genetic population structure and in the estimation of migration between sites. An excess of homozygotes at esterase loci as observed in our samples could have resulted, among other hypotheses, from a pooling of sex or age-classes characterized by different gene frequencies. This effect has been invoked to explain genotypic disequilibrium in other fish species such as Pacific hake (*Merluccius productus*) (Utter et al. 1970), mummichog (*Fundulus heteroclitus*) (Mitton and Koehn 1975), and Atlantic mackerel (*Scomber scombrus*) (Smith et al. 1981).

It is possible that selective phenomena are involved in the directional changes in gene frequencies observed between age-classes within a sample, indicating an association of different alleles to viability with respect to constant environmental stress factors. Kirpichnikov (1981) argued that conclusions about the presence of selection could be drawn by a comparison of the different age groups of one and the same population, preferably in the space of one generation, or by direct selection experiments. Among environmental factors known to exert a selective pressure on fish, temperature has been positively correlated with the genetic variability of a locus coding for malic enzyme in Atlantic salmon (*Salmo salar*) (Verspoor and Jordan 1989) and of esterase loci in the crested blenny (*Anoplarchus purpurescens*) (Johnson 1977), New Zealand snapper (*Chrysophrys auratus*) (Smith 1979), Atlantic mackerel (Smith et al. 1981), and Arctic char (*Salvelinus alpinus*) (Dempson et al. 1988), suggesting possible direct or indirect effects of selection on these loci. Selection due to local environmental factors probably inflates the estimate of genetic divergence between samples. The action of selection could then explain the significant heterogeneity which was observed among the allelic frequencies of our samples, notably at the locus coding for malic enzyme, even in the presence of a high level of gene flow. The east–west geographic differentiation observed in this study should be interpreted with caution, since it may, at least partially, result from spatially varying selective environmental pressures.

The possible effect of selection on protein loci diminishes the pertinence of their use as markers in stock identification studies. It may be of greater interest, however, in the understanding of major processes involved in the evolution of the

TABLE 9. Comparison of Wright hierarchical analysis performed on samples grouped according to NAFO divisions and according to their genetic relatedness herein estimated. Sam-area = variance corresponding to sample differentiation within areas; Sam-tot = within sample variance relative to the total variance over all samples; Area-tot = variance corresponding to the differentiation of suggested areas.

	NAFO divisions			Observed groups		
Locus	Sam-area	Sam-tot	Area-tot	Sam-area	Sam-tot	Area-tot
MEP	0.009	0.004	−0.005	0.000	0.008	0.008
MDH-1	0.003	0.001	−0.002	0.000	0.003	0.003
MDH-2	0.006	0.003	−0.003	0.000	0.006	0.006
GPI-1	0.010	0.005	−0.005	0.010	0.006	−0.005
GPI-2	0.001	0.000	−0.001	0.000	0.001	0.001
PGM	0.004	0.002	−0.002	0.004	0.003	−0.002
MPI	0.007	0.003	−0.003	0.002	0.005	0.003
IDDH	0.011	0.007	−0.003	0.011	0.007	−0.004
EST-1	0.000	0.000	0.000	0.000	0.001	0.001
EST-2	0.000	0.001	0.001	0.000	0.002	0.002
Mean	0.003	0.002	−0.001	0.001	0.003	0.002

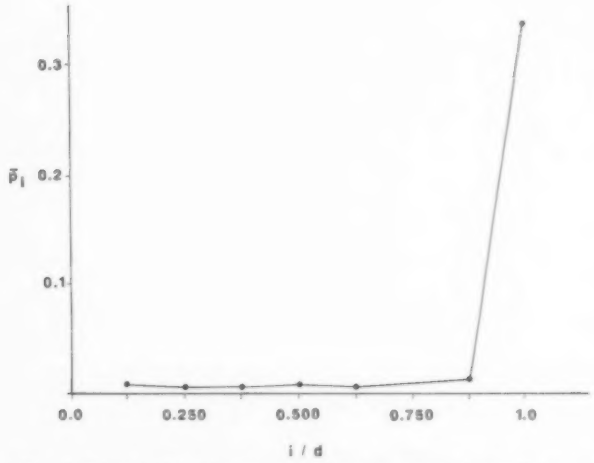

FIG. 6. Relationship between conditional average frequency, $\bar{p}_i$, of alleles, and their incidence, i/d, in capelin populations, where i is the total number of samples where an allele appears and d is the total number of samples.

studied species. Selective processes may be partly responsible, in the short term, for recruitment variability due, for example, to differential survival of genotypes in early life stages (Mork and Sundness 1985).

The present results stress the importance of analysing every source of variation and of relating it to the overall pattern before drawing conclusions about intersample heterogeneity. The evaluation of the status of the analysed loci with respect to selection is an essential step in the study of genetic population structure.

Acknowledgments

The authors thak J. P.Allard, M. Aparicio, and R. Morneau for sampling and carefully collecting data and tissues from the studied capelin, S. Levesque, A. Sirois, and D. Archambault for providing technical assistance with the electrophoresis work, and L. Corriveau for drawing the figures. K. Somers kindly provided a revised version of his constrained principal component analysis. S. Ochman, Y. de Lafontaine, and two anonymous reviewers read the manuscript and made helpful comments. G. Lobb kindly volunteered the time and patience to make the manuscript more readable to all. The research was funded by the Department of Fisheries and Oceans.

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Ingestion of Small-Bodied Zooplankton by Zebra Mussels (*Dreissena polymorpha*): Can Cannibalism on Larvae Influence Population Dynamics?

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The zebra mussel *Dreissena polymorpha* established populations in western Lake Erie in 1986 and achieved densities exceeding 3.4×10^5 individuals·m^{-2} during 1990. We assessed apparently incidental predation on Lake Erie and Erindale Pond zooplankton by adult mussels. *Dreissena* larvae and small rotifers (*Polyarthra* spp., *Keratella* spp., *Trichocerca*) sustained moderate to high predatory mortality whereas larger taxa (*Bosmina*, *Scapholeberis*) were invulnerable to predation. Larval *Dreissena* almost always sustain >99% mortality in European lakes. While mortality has been ascribed primarily to lack of suitable settling substrate and unfavourable environmental conditions, it may be confounded by larval predation by adults. We demonstrate using STELLA®-modelling that with a larval mortality rate of 99%, settled mussel densities observed in western Lake Erie during 1990 would not be achieved until at least 1994. A model that combines a lower rate (70%) of abiotic mortality with larval predation by adult mussels correctly predicts 1990 mussel densities. This model of *Dreissena* population growth is also consistent with larval settlement patterns in Lakes Erie and St. Clair and some European systems.

Les populations de dreissena polymorphe (*Dreissena polymorpha*) déjà établies dans la partie ouest du lac Érié en 1986 atteignaient des densités supérieures à $3,4 \times 10^5$ individus par mètre carré au cours de 1990. Nous avons évalué la prédation du zooplancton du lac Érié et de l'étang Erindale par les dreissenas adultes. Les larves des dreissenas et les petits rotifères (*Polyarthra* spp., *Keratella* spp., *Trichocerca*) présentaient une mortalité par prédation variant de moyenne à élevée tandis que les plus gros taxons (*Bosmima*, *Scapholeberis*) résistaient à toute prédation. Les larves de *Dreissena* des lacs européens présentent presque toujours un taux de mortalité supérieur à 99 %. La mortalité a surtout été attribuée à l'absence d'un substrat de fixation adéquat et à des conditions environnementales défavorables, mais elle peut être confondue avec celle découlant de la prédation des larves par les adultes. Nous avons montré, à l'aide d'un modèle STELLA$_{md}$, que les densités de dreissenas fixées notées en 1990 n'auraient été atteintes qu'en 1994 dans le lac Érié si le taux de mortalité avait été de 99 %. Les densités réelles de 1990 n'ont pu être prévues qu'en utilisant un modèle dont le taux de mortalité abiotique par prédation par les adultes était plus faible (70 %). Ce modèle de la croissance des populations de *Dreissena* est cohérent avec les allures de fixation des larves décelées dans les lacs Érié et Ste-Claire et dans certains bassins hydrographiques européens.

Received April 22, 1991
Accepted July 19, 1991
(JB008)

Reçu le 22 avril 1991
Accepté le 19 juillet 1991

The composition of biological communities in the St. Lawrence Great Lakes has been profoundly altered by the introduction of exotic species. The zebra mussel *Dreissena polymorpha* (Pallas) is one of the most recent and potentially destructive species to invade these ecosystems. *Dreissena* was first observed in North America in Lake St. Clair and western and central Lake Erie during 1988 (Hebert et al. 1989). Based on mussel size distributions in Lake St. Clair, Hebert et al. (1991) suggested that populations first became established during 1986.

Dreissena is unique among North American freshwater bivalves in possessing free-swimming veliger larvae and byssate adults. These unusual life history characteristics have facilitated rapid dispersal and the exploitation of unused solid, benthic substrates. Hebert et al. (1991) described 1000-fold increases in settled mussel densities in Lake St. Clair between 1986 and 1989. Populations inhabiting reefs in western Lake Erie expanded from a maximum mean density of 3.2×10^4 individuals·m^{-2} in May 1989 to 3.42×10^5 individuals·m^{-2} in October 1990 (Fig. 1).

Female and male *Dreissena* synchronously release eggs and sperm into the water column where fertilization takes place. Veligers remain free-swimming for periods of 8–45 d, during which time they grow from ~70 to 250 μm. Veligers utilize a ciliated velum for propulsion and food collection. Individuals between 200 and 250 μm body length undergo metamorphosis to become postveligers; in the process the velum is replaced by siphons, the characteristic bivalve foot and shells develop, and

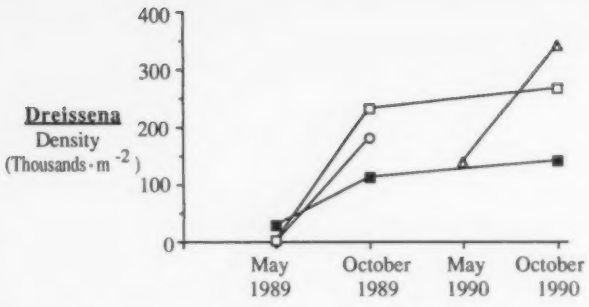

FIG. 1. Mean estimated population densities of *Dreissena polymorpha* inhabiting reefs west (open symbols) and southeast (solid symbols) of Pelee Island, Ontario, in the western basin of Lake Erie. Individuals ranged between 1 and 29 mm in maximum shell length.

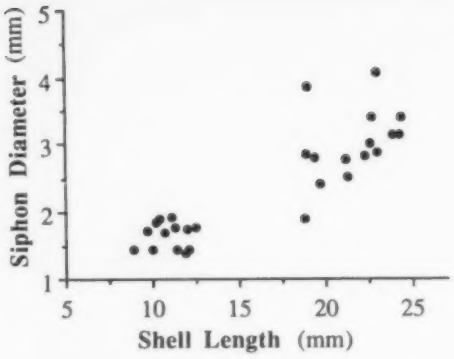

FIG. 2. Relationship between exterior inhalant siphon diameter and *Dreissena* shell length. Pearson's correlation coefficient, $r = 0.87$.

the animals settle from the plankton and attach to benthic substrates (Lewandowski 1982; Sprung 1989; reviewed in Mackie et al. 1989).

Overall dynamics of settled *Dreissena* populations appear to be governed primarily by the severity of mortality between planktonic veliger and settled subadult phases. Mortality between these phases almost always exceeds 99% (Stańczykowska 1977; Walz 1978a; Lewandowski 1982; Sprung 1989). Veliger and postveliger mortality has been attributed to a lack of suitable settling substrate, unfavourable oxygen or thermal conditions, and predation by larval fish or invertebrates, but their respective intensities have not been determined (Stańczykowska 1977; Lewandowski 1982; Lewandowski and Ejsmont-Karabin 1983). Moreover, these sources of mortality may be confounded by predation by adult *Dreissena*. For example, adult marine bivalves have been reported to consume both conspecific and heterospecific larvae (see Bayne 1964; André and Rosenberg 1991).

The ability of adult *Dreissena* to prey on planktonic veligers and settling postveligers, and thereby influence recruitment dynamics, has received scant attention. Zooplankton has been infrequently reported in *Dreissena* diets, presumably because of their relatively large size. Large particles irritate tentacles lining the inhalant siphon and are rejected. Other particles may also be sorted and rejected by the labial palps, mouth, and stomach (Morton 1969). Adult *Dreissena* have been reported to ingest some large food items, nevertheless. Ten Winkel and Davids (1982) indicated that well-fed adult *Dreissena* preferred algal foods smaller than 50 μm, while starved individuals fed nonselectively and ingested diatoms as large as 750 μm. Mikheyev (1967) reported that particles between 80 and 450 μm, including microzooplankton, were ingested by *Dreissena*. The clearest evidence of zooplankton predation by *Dreissena* was provided by Shevtsova et al. (1986) in a series of laboratory and field experiments. They determined that adult *Dreissena polymorpha* and *Dreissena bugensis* consumed a wide array of microzooplankton up to 400 μm in body length.

In this study we assess the ability of *Dreissena polymorpha* to prey on populations of microzooplankton from Lake Erie and Erindale Pond. In addition, we propose a model that relates *Dreissena* population growth in western Lake Erie to the density of predatory adults. Our purpose in constructing the model was to demonstrate that *Dreissena* population growth patterns in the lake are inconsistent with predictions based on European experiences (i.e. >99% density-independent larval mortality).

Materials and Methods

Population densities and size distributions of *Dreissena* inhabiting reefs in the western basin of Lake Erie were estimated by collecting representative rock samples. Samples were collected from reefs west and southeast of Pelee Island. Six to 12 collections were made per reef for each date.

The relationship between *Dreissena* maximum shell length and exterior inhalant siphon diameter was explored using live animals maintained in laboratory cultures. Shell length and siphon diameter were measured to the nearest 0.01 mm with a Fowler ultra-cal II digital caliper and microcomputer-based caliper measurement system (Sprules et al. 1981), respectively. Inhalant siphon diameter of *Dreissena* increases with shell length ($r = 0.87$, $n = 28$; Fig. 2) and can exceed the body size of many species of Great Lakes zooplankton by an order of magnitude (Stemberger 1979).

Predation Trials

Vulnerability of individual zooplankton species to predation by adult *Dreissena* was initially assessed in short-term trials in 50-mL beakers. *Dreissena* was collected from submerged littoral rocks in Lake Erie near Wheatley, Ontario, and maintained in aerated laboratory aquaria at 20°C in a culture medium of glass-fibre-filtered (Whatman 934-AH) Erindale Pond (Erindale College, Mississauga, Ontario) water supplemented every 2 d with ~0.8 μg *Cryptomonas* sp.·mL^{-1} (9×10^3 cells·mL^{-1}). Zooplankton for all experiments was collected from 1–2 m depth with a 30-L Schindler–Patalas trap outfitted with a 41-μm Nitex mesh sock. Lake Erie zooplankton was maintained in *Cryptomonas*-supplemented laboratory cultures prior to experiments. All experimental adult mussels were of the same approximate size (mean shell length ± SD = 2.25 ± 0.23 cm) and were acclimated in test beakers for 2 h prior to predation trials. Experiments were conducted in 50 mL of culture medium supplemented with 0.45 μg dry weight *Cryptomonas* sp.·mL^{-1} to simulate natural food conditions. Zooplankton was incubated in beakers, in triplicate, with zero (control) or one adult mussel for 2 h, after which time the mussel was removed and zooplankton survivorship assessed (experiment 1). Experiments were conducted with the rotifers *Keratella crassa* (mean body length ± SD = 208 ± 13 μm), *Polyarthra remata* (82 ± 9 μm), and *Trichocerca multicrinis* (189 ± 17 μm), *Dreissena* veligers (170 ± 40 μm), and the cla-

docerans *Bosmina longirostris* (312 ± 62 μm) and *Scaphloeberis kingi* (647 ± 124 μm). Fifty rotifers or mussel veligers or 25 cladocerans were used to initiate trials. Wastes expelled by, and digestive tracts of, mussels were examined for the presence of zooplankton. The effect of *Dreissena* on zooplankton survival was assessed for each species using Bonferroni-corrected *t*-tests on $\ln(x + 1)$-transformed abundance data for control and experimental populations.

A second laboratory experiment was conducted to assess the capability of adult *Dreissena* to suppress zooplankton from Erindale Pond (experiment 2). Macrozooplankton was removed to preclude interactions with microzooplankton by straining pond water through 363-μm Nitex mesh. Experiments consisted of placing 3 L of pond water containing microzooplankton, concentrated to 150% of pond density, in 4-L jars with zero (control), one, or three adult *Dreissena*. These *Dreissena* densities are well within the range of effective densities found in western basin of Lake Erie during 1990 (Leach 1991). All treatments were replicated three times. Experiments were conducted in a controlled environment chamber at 20°C with photoperiod of 15 h light : 9 h dark. Experiments lasted 24 h, after which time mussels were removed from test containers and zooplankton concentrated and fixed with 4% sucrose–formalin. Zooplankton was subsampled, settled overnight, and counted at 63× magnification. Differences in zooplankton densities between treatments were analyzed with ANOVA and Scheffe's multiple comparisons tests performed on $\ln(x + 1)$-transformed abundance data (Day and Quinn 1989).

Two similar experiments were conducted in Lake Erie adjacent to Wheatley, Ontario. In an experiment conducted between 22 and 23 August 1990, 3.7 L of lake water was filtered through 363 μm Nitex mesh to remove macrozooplanton, and placed into 4-L bottles with zero, two, or four adult *Dreissena* (experiment 3). The bottles were sealed and tethered in wire-mesh baskets at 5 m for 24 h, after which time zooplankton and *Dreissena* were fixed as above. Three control zooplankton samples were fixed at the outset of the experiment to test for the presence of a bottle effect. While all other treatments were replicated four times, strong seas reduced the number of recovered replicates to two for each treatment.

In an otherwise identical experiment conducted in Lake Erie between 9 and 11 July 1990, zooplankton was incubated with zero or six adult *Dreissena*. *Cryptomonas* (0.62 μg dry weight·mL^{-1}) was added to one *Dreissena* treatment to supplement ambient food resources and reduce exploitation competition between *Dreissena* and *Polyarthra*, the numerically dominant zooplankter in the lake (experiment 4). The second *Dreissena* treatment and control zooplankton containers were incubated with ambient food and, as a volume control, an equivalent quantity of algal growth medium as that placed in the treatment with supplemented food. Zooplankton and *Dreissena* were preserved after 50 h. *Polyarthra* densities were $\ln(x + 1)$-transformed prior to statistical analysis with an ANOVA test.

Dreissena prey-based clearance rates (CR) and ingestion rates (IR) were calculated for each species for each experiment as

$$CR = V(\ln[E_0/E_t] - \ln[C_0/C_t])/tn$$
$$IR = CR \cdot C_0$$

where V was the experimental volume, E_0 and E_t were the initial and final prey densities in the experimental vessels, respectively, C_0 and C_t were the initial and final prey densities in the controls, respectively, t was the experimental period, and n was the predator number (modified from Reeders et al. 1989).

We compared the potential predation impact of *Dreissena* on rotifers with impacts reported for other invertebrate predators in the Great Lakes. Predation impact refers to the per capita mortality rate imposed on rotifer prey by the predator population in one day and is calculated as the product of predator volume density (predators per cubic metre) and predator clearance rate (litres per predator per day). Settled *Dreissena* densities (predators per square metre) were converted to volume densities (predators per cubic metre) by assuming that the overlying water column was well mixed and 7 m deep. We conservatively estimate that zooplankton was preyed on only by mussels larger than 2 cm, which averaged 5000 individuals·m^{-2} (i.e. 714.3 individuals·m^{-3}) on reefs in western Lake Erie during the fall of 1990 (Leach 1991). We used the median rotifer clearance rate from all our experiments (0.99 L·predator^{-1}·d^{-1}) for the predation impact calculation. Densities and rotifer-based clearance rates of other predators were derived from the literature.

Population Modelling[1]

We used STELLA® software (High Performance Systems, Inc. 1988) to construct two models of *Dreissena* population growth in the western basin of Lake Erie. The models differ only with respect to the sources and intensities of larval mortality. The first model, which for clarity we define as the noncannibalistic model (see Appendix 1), assumes a constant density-independent mortality rate between veliger and settled adult phases of 99% due to inhospitable settling substrate (e.g. mud, sand), unsuitable chemical (e.g. oxygen) or thermal conditions, and from fish and invertebrate predation (see Stańczykowska 1977; Lewandowski 1982; Sprung 1989). The second model, termed the composite model (Appendix 1), combines a constant density-independent mortality of 70% with density-dependent mortality due to predation by conspecific adults so that the total mortality rate can exceed 99%. Mussel populations in each model consist of four life history stages, individuals within which have characteristic fecundity functions, and, in the composite model, cannibalism functions. The compartments represent subpopulations of (1) veligers, (2) reproductive but nonpredacious 1-yr-old adults, and (3 and 4) reproductive and predacious 2- and 3-yr-old adults. All individuals die by the end of their third year, as apparently occurs in Lake St. Clair (Mackie 1990). Mortality rates between adult compartments were parameterized using the fall 1990 population size structure in western Lake Erie (J. Leach, unpubl. data) (see Appendix 1). Mortality between age-classes is not ascribed to specific agents, but apparently can result from both density-dependent and density-independent factors (Stańczykowska 1977; Hebert et al. 1991). Age-specific fecundity (Appendix 1) was parameterized using European literature values (Stańczykowska 1977; Walz 1978b).

The composite model incorporates separate predation functions for 2- and 3-yr old adult mussels feeding on veligers and postveligers. Planktonic veligers may be brought into contact with, and be ingested by, 2- and 3-yr-old mussels due to mixing of the water column. Settling postveligers may become entrained in feeding currents of adult mussels and consumed. Predation rates were modelled as negative functions of adult

[1]See Note Added in Proof.

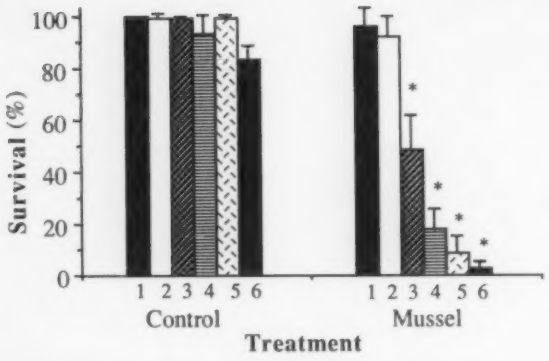

FIG. 3. Mean (+1 SE) densities of the cladocerans *Bosmina longirostris* (1) and *Scapholeberis kingi* (2), *Dreissena* veligers (3), and the rotifers *Trichocerca multicrinis* (4), *Keratella crassa* (5), and *Polyarthra remata* (6) in the absence and presence of one adult *Dreissena* (experiment 1). *Significant *t*-test ($p < 0.01$) difference between density in control and mussel treatment.

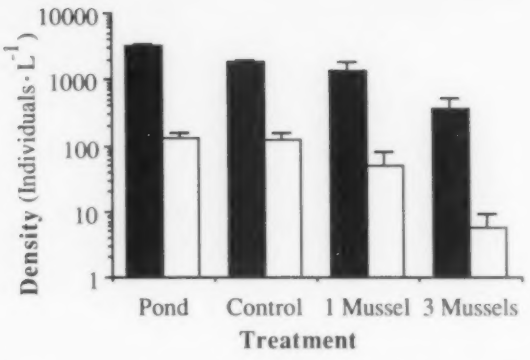

FIG. 4. Population densities (mean +1 SE; $n = 3$) of *Polyarthra remata* (solid) and *Keratella cochlearis* (open) in the presence of zero (control), one, or three adult *Dreissena* (experiment 2). Plankton samples were also preserved prior to the experiment (Pond).

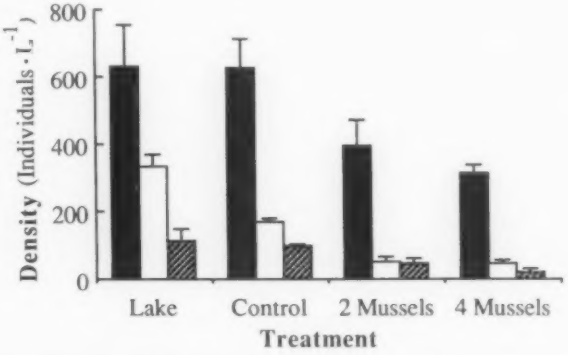

FIG. 5. Population densities of Lake Erie *Dreissena* veligers (solid), *Polyarthra* spp. (open), and *Keratella* spp. (hatched) in samples with zero (control), two, or four adult *Dreissena* (experiment 3). Zooplankton was also preserved prior to the experiment (Lake). Each bar represents the mean (+1 SE) of three (lake) or two (control, mussel treatments) replicate samples.

density (Appendix 1) because refiltration of water, from which larvae have already been strained, can be appreciable in the benthic boundary layer when sessile mussel densities are high (Fréchette et al. 1989). The exact nature of the predation rate curves is difficult to establish because postveliger susceptibility to cannibalism varies inversely with distance above mussel beds.

Population densities within each compartment were calculated based on a uniform mussel distribution over 334 km^2 of habitable substrate (gravel and bedrock) in the western basin (derived from Hartman 1973). Our models assume an initial veliger inoculum of 5×10^8 individuals, based on a ballast volume of 10^7 L containing 50 veligers·L^{-1}, dumped entirely into western Lake Erie. Our modelling approach is conservative (i.e. it favours the noncannibalistic model) for a number of reasons. First, it is very unlikely that all of the ballast containing the inoculum was actually dumped into Lake Erie because mussels were first observed in Lake St. Clair. It is also unlikely that the veliger inoculum was as large as modelled because it assumes a large ballast volume and a high density of veligers surviving transit in ballast tanks. Finally, the noncannibalistic model assumes a larval mortality rate (99%) considerably lower than the values commonly estimated for European systems (Stańczykowska 1977; Lewandowski 1982; Sprung 1989). We assessed the sensitivity of both noncannibalistic and composite models by varying reproductive schedules and larval mortality rates and observing resultant time required to reach 1990 density from initial density (see Appendix 1). The models iterate once per year (i.e. individuals spend 1 yr in each age-class compartment) using Euler's integration method.

Results

Laboratory Experiments

All small zooplankton species incubated with adult *Dreissena* sustained significant (*t*-tests, $p < 0.01$; Fig. 3) mortality in the short-term laboratory experiments whereas densities of large-bodied species were unaffected (*t*-tests, $p > 0.05$). The rotifer *Polyarthra remata* was suppressed most severely (<5% survival) by *Dreissena*. *Keratella crassa* also sustained intense mortality (9% survival). *Dreissena* digestive tracts contained *Keratella* loricae at the end of the experiment, verifying predation as the suppressive mechanism. Survival rates of experimental *Trichocerca similis* and *Dreissena* veligers were 17% and 49%, respectively, while those of the cladocerans *Bosmina longirostris* and *Scapholeberis kingi* were greater than 90% (Fig. 3). Considering the small volume of the experimental vessels, these data are most useful in a broad consideration of differential zooplankton susceptibility to predation. They are, however, corroborated by experiments conducted in the laboratory and field using much larger volumes and realistic *Dreissena* densities.

The late-summer Erindale Pond zooplankton community was dominated numerically by *Polyarthra remata* and *Keratella cochlearis*. Densities of both species were significantly (experiment 2; ANOVA tests, $p < 0.05$) lower in bottles with three mussels than in those lacking mussels (Fig. 4), while densities in bottles with one mussel were not statistically different from controls (Scheffe's tests, $\alpha = 0.05$). The density of *Polyarthra* was also influenced by the experimental procedure, as mean density of control populations preserved at time 0 ("pond") was significantly greater than that of control

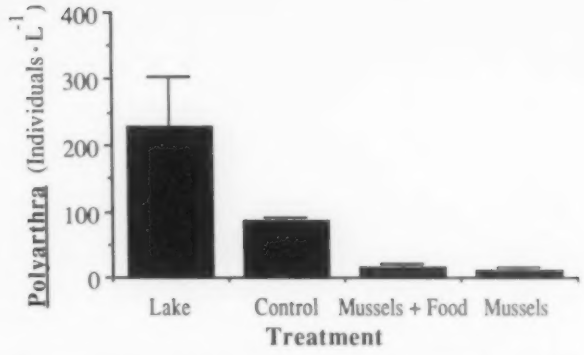

FIG. 6. Densities of Lake Erie *Polyarthra* spp. after 50 h in treatments with zero (control) or six (mussels + food, mussels) adult *Dreissena* (experiment 4). Control samples were also preserved immediately prior to the experiment (lake). Food in the mussels + food treatment was supplemented with 0.63 μg *Cryptomonas* sp.·mL^{-1}. Each bar is the mean (+1 SE) of three (lake, control), four (mussels + food), or two (mussels) replicate samples.

populations preserved at the end of the experiment (Scheffe's test, $\alpha = 0.05$).

Field Experiments

The microzooplankton community of Lake Erie near Wheatley, Ontario, was dominated numerically by *Dreissena* veligers, *Polyarthra* spp., and *Keratella* spp. during late August 1990. Each of these taxa experienced graded predatory losses in relation to *Dreissena* density (experiment 3; Fig. 5). Although veliger mortality tended to increase with *Dreissena* density, differences in density were not significant (ANOVA, $0.10 > P > 0.05$) because of low statistical power associated with small sample sizes. *Polyarthra* density was significantly (ANOVA, $p < 0.001$) lower in bottles containing *Dreissena*, but differences between treatments with two and four mussels were not significant (Scheffe's test, $\alpha = 0.05$). The density of *Keratella* varied in a similar manner, although differences among individual means were not significant (Scheffe's tests, $\alpha = 0.05$).

Polyarthra spp. dominated the Lake Erie plankton community numerically earlier in the summer at the same locality (experiment 4). *Polyarthra* densities were significantly lower in bottles with *Dreissena* than in controls (ANOVA, $p < 0.005$). Food supplementation did not decrease the effect of *Dreissena* (Fig. 6), as *Polyarthra* densities were only slightly lower in containers incubated with ambient food. Exploitation competition appeared to play a minor role, if any, in the suppression of *Polyarthra*.

Dreissena clearance and ingestion rates on Lake Erie and Erindale Pond zooplankton were strongly related to prey size (Table 1). Ingestion rates were highest on rotifers, intermediate on *Dreissena* veligers, and lowest on crustaceans. Predation rates on *Polyarthra* were always higher than those on *Keratella* within an experiment. Ingestion rates of *Polyarthra* were also quite variable, reflecting moderate differences among experiments in calculated clearance rates (Table 1) and large differences in initial zooplankton density.

Large *Dreissena* (>2 cm) had an effective average density of 714 individuals·m^{-3} in western Lake Erie and a median rotifer-based clearance rate of 41.2 mL·predator^{-1}·h^{-1} (0.99 L·predator^{-1}d^{-1}) (Table 2). At these rates, adult *Dreissena* can impose a per capita death rate of up to 0.71·d^{-1} on vulnerable rotifer prey, assuming complete mixing of the water column. This predatory potential exceeds that of other invertebrate predators by more than an order of magnitude (Table 2).

Population Modelling

Dreissena densities observed during 1990 in western Lake Erie could not have been achieved if total mortality equalled or exceeded 99% (noncannibalistic model), even if high repro-

TABLE 1. Clearance rates and ingestion of *Dreissena* on Lake Erie and Erindale Pond microzooplankton. Experiment 4a, ambient food level; experiment 4b, ambient food level supplemented with ~0.62 μg *Cryptomonas* sp. dry weight·mL^{-1}.

Experiment	Zooplankton taxon	*Dreissena* (number·jar^{-1})	Volume (L)	Time (h)	*Dreissena* Clearance rate (mL·ind.$^{-1}$·h^{-1})	*Dreissena* Ingestion rate (prey·ind.$^{-1}$·h^{-1})
1	*Bosmina longirostris*	1	0.05	24	1.8	0.9
1	*Scapholeberis kingi*	1	0.05	24	1.0	0.5
1	*Dreissena* veligers	1	0.05	24	17.7	17.7
1	*Trichocerca multicrinis*	1	0.05	24	41.2	41.2
1	*Keratella crassa*	1	0.05	24	61.0	61.0
1	*Polyarthra remata*	1	0.05	24	86.0	86.0
2	*Keratella cochlearis*	1	3	24	108.7	14.6
2	*Keratella cochlearis*	3	3	24	126.4	17.0
2	*Polyarthra remata*	1	3	24	65.6	203.3
2	*Polyarthra remata*	3	3	24	34.7	107.5
3	*Dreissena* veligers	2	3.7	24	13.4	11.3
3	*Dreissena* veligers	4	3.7	24	13.4	8.5
3	*Polyarthra* spp.	2	3.7	24	45.8	15.2
3	*Polyarthra* spp.	4	3.7	24	25.6	8.5
3	*Keratella* spp.	2	3.7	24	30.0	3.4
3	*Keratella* spp.	4	3.7	24	29.3	3.4
4a	*Polyarthra* spp.	6	3.7	50	24.5	5.6
4b	*Polyarthra* spp.	6	3.7	50	20.0	4.6

TABLE 2. Potential impacts of Great Lakes invertebrate predators on planktonic rotifers based on typical predator densities and clearance rates. Clearance rate is the volume of water from which an individual predator removes all prey in 1 d. Impact is the per capita prey death rate exerted by the predator population. Source key: 1 = Nero and Sprules (1986); 2 = Grossnickle (1990); 3 = Fero and Riessen (1990); 4 = Vanderploeg et al. (1990); 5 = this study. *Dreissena* volumetric density was calculated based on 5000 adult mussels·m^{-2} and a mixed water column 7 m deep.

Predator	Predator density (ind.·m^{-3})	Rotifer-based clearance rate (L·predator^{-1}·d^{-1})	Impact (d^{-1})	Source
Mysis relicta	4.3	4.3	0.02	1
Limnocalanus macrurus	61	0.16	0.01	1
Bythotrephes cedestroemi	23	0.26	<0.01	2, 3, 4
Senecella calanoides	93	0.44	0.04	1
Dreissena polymorpha	714.3	0.99	0.71	5

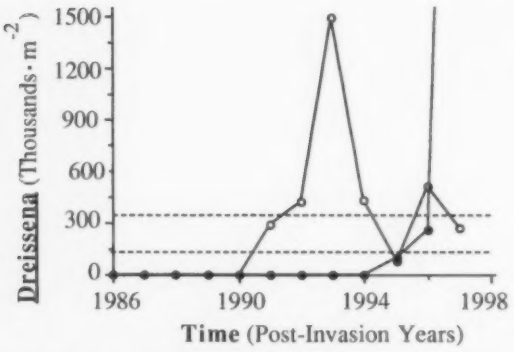

FIG. 7. Projected population abundance of *Dreissena* in western Lake Erie according to composite (open circles) and noncannibalistic (closed circles) models. The range of mean densities observed on reefs during 1990 is denoted by the broken horizontal lines (Fig. 1). Time of invasion is assumed to be 1986. Both models assume fecundity values of 1×10^4, 2×10^4, and 3×10^4 eggs·female^{-1}·yr^{-1} for 1-, 2-, and 3-yr-old adults, respectively. Total larval mortality rate = 99% in the noncannibalistic model. Initial larval mortality rate = 80% in the composite model.

ductive schedules are utilized (Fig. 7; Table 3). Indeed, the noncannibalistic model does not predict 1990 densities until at least 1994 when total larval mortality equals or exceeds 99% (Table 3). These results are insensitive to variation in initial veliger inoculum, as the time to 1990 density is decreased by only 1 yr if the inoculum is increased by an order of magnitude (5×10^9 individuals). By contrast, the composite model correctly projects 1990 densities if total larval mortality is initially set at 70% (Table 3; Fig. 7). Larval mortality did not exceed 99% in the composite model until the combined densities of 2- and 3-yr-old adults exceeded 8×10^4 individuals·m^{-2}.

Discussion

Our study indicates that some species of zooplankton may experience high mortality rates in shallow, well-mixed regions of the Great Lakes which support large populations of adult *Dreissena*, including western Lake Erie and southern Lake St. Clair. Small-bodied, weak-swimming species of rotifers and *Dreissena* veligers appear particularly susceptible to entrainment in filtering currents generated by adult *Dreissena* (Fig. 3; Table 1). Our findings are generally consistent with those of Shevtsova et al. (1986) who reported that adult *Dreissena* consumed many small zooplankton species including the rotifers *Keratella quadrata*, *Polyarthra vulgaris*, and *Euchlanis dilatata*, *Dreissena* veligers, protozoans, and cyclopoid copepodids. However, whereas we found that relatively large zooplankton (*Bosmina*, *Scapholeberis*) were invulnerable to ingestion by *Dreissena* (Fig. 3), Shevtsova et al. (1986) reported that some large species (*Bosmina*, *Podon*) were vulnerable while others (*Chydorus*, *Alona*) were not.

Maximum *Dreissena* prey size may be constrained by the effective diameter of the inhalant siphon. For example, contact between *Scapholeberis* and tentacles lining the inhalant siphon elicited a rapid response from the mussel in which water intake ceased and the siphon was temporarily withdrawn. Smaller species of zooplankton flowed into the mussel's mantle cavity without stimulating siphonal tentacles. The minimum size of *Dreissena* capable of ingesting zooplankton has not been determined but is probably correlated with the inner diameter of the inhalant siphon and mussel shell length. Shevtsova et al. (1986) determined that *Dreissena* as small as 18–20 mm consumed zooplankton. It is also possible that some zooplankton drawn into the mantle cavity may be expelled in pseudofaeces, as has been reported for the marine mussel *Mytilus edulis* (Bayne 1964).

Prey swimming ability and vertical location in the plankton may also influence predation rates. *Polyarthra* is capable of escaping from weak flow currents only, such as those generated by feeding *Daphnia*, by rapidly beating its ventral paddles (Gilbert 1987). This tactic was insufficient to propel some individuals from the inhalant current of adult *Dreissena* despite repeated escape attempts. By contrast, copepods and other zooplankton capable of strong swimming bursts are unlikely to become trapped in mussel feeding currents (H. J. MacIsaac, pers. obs.). Vertical separation of planktonic zooplankton and benthic *Dreissena* may also reduce predation rates, particularly when the water column is stratified or poorly mixed. This problem is unlikely to affect predation rates on postveligers because they must sink from the plankton during settlement, and possibly encounter adults in the process (see Bayne 1964).

Settled *Dreissena* may suppress Lake Erie zooplankton through a combination of exploitative competition and predation. Between 1988 and 1990, water clarity in the western basin almost doubled, settled mussel populations increased from 400 to 341 000 individuals·m^{-2}, and chlorophyll *a* and particulate organic carbon levels declined (Leach 1991). Based on fall 1990 *Dreissena* size–frequency distributions in western Lake Erie and associated literature-derived clearance rates, H. J. MacIsaac and W. G. Sprules (unpubl. data) calculated that sessile

TABLE 3. Variation in predicted time to 1990 *Dreissena* density for noncannibalistic and composite models in relation to age-specific fecundity and total larval mortality rate. High and low reproductive values were derived from European studies (Walz 1978b; Stanczykowska 1977), as were larval mortality rates (see Discussion). Western Lake Erie fall 1990 population survey data (Leach 1991) were used to establish age structure: populations consisted of 74, 22, and 4% of adult age-classes 1, 2, and 3, respectively. All models assume a veliger inoculum of 5×10^8 individuals (see Materials and Methods).

Model	Larval mortality rate (% of veligers produced)	Fecundity (eggs·female^{-1}·yr^{-1}) Age 1	Age 2	Age 3	Year 1990 mussel density reached
Noncannibalistic		10^4	2×10^4	3×10^4	
	99				1996
	99.5				1998
		10^4	2×10^5	10^6	
	99				1994
	99.5				1996
Composite		10^4	2×10^4	3×10^4	
	70				1990
	80				1991
	90				1992
		10^4	2×10^5	10^6	
	70				1990
	80				1991
	90				1991

zebra mussel populations may filter the water column more than 18 times per day. These calculations indicate that the western basin may be experiencing an unprecedented diversion of energy from pelagic to benthic food webs and corroborate the high ingestion rates and predation impacts observed in this study (Tables 1 and 2). Comparative data describing microzooplankton densities before and after *Dreissena* establishment in European systems are lacking. However, Shevtsova and Kharchenko (1981) commented that low stocks of bacterioplankton, phytoplankton, and zooplankton in the North-Crimean Canal appeared to have been caused by the filtering activities of large populations of *Dreissena*. Knowledge of these impacts is essential considering the dietary dependence of early life history stages of commercial Lake Erie fishes (e.g. *Perca*) on microzooplankton (Treasurer 1990).

Most of our microzooplankton-based *Dreissena* clearance rates were less than 100 mL·individual^{-1}·h^{-1} (Table 1). These rates are consistent with those from some previous investigations in which natural phytoplankton or other small particles were used as tracers (see Kryger and Riisgård 1988) and indicate that *Dreissena* may have been feeding relatively nonselectively on microzooplankton. *Dreissena* had unusually high clearance rates on *Keratella* in experiment 2 (Table 1); these values resulted from the depletion of *Keratella* in experimental vessels and from the sensitivity of the clearance rate equation to prey depletion.

Mortality in *Dreissena* populations is generally most concentrated between the planktonic veliger and settled subadult phases. Because individual females are capable of producing up to 10^6 eggs per year (Walz 1978b), mortality of larvae must approach 100% in stable or near-stable populations. The determinants of larval mortality have remained elusive despite the practical significance of the problem. While our study clearly implicates intraspecific predation as a regulatory mechanism, European workers have focused primarily on the availability of suitable settling substrate (see Stańczykowska 1977; Lewandowski 1982). The available evidence is not entirely consistent with this hypothesis, however. Assuming that veligers have a uniform horizontal distribution in the water column, a mortality rate exceeding 99% implies that less than 1% of benthic habitats are suitable for colonization by *Dreissena*, an unlikely possibility in most lakes. Indeed, veligers are often concentrated in belts above benthic adults (Stańczykowska 1977) and thus may actually have a higher probability of settling on suitable substrate than if their horizontal distribution were uniform. Lewandowski (1982) calculated that mortality to the settling stage amounted to 99.4% in Lake Kolowin, Poland, despite the abundance of suitable settling substrate (21.8% of surface area). As much as 56.4% of benthic substrate in Lake Majcz Wielki, Poland, was suitable for settlement of postveligers, yet mortality amounted to 95.8% of the initial veliger cohort (Lewandowski 1982). Postveliger densities in western Lake Erie declined by 44% between 1989 and 1990 despite a 75-fold increase in settled mussel density (Garton and Haag 1990) and a threefold increase in the density of planktonic veligers (Leach 1991). If recruitment was determined simply by the amount of total substrate in the basin, 1990 settlement values should have greatly exceeded those for 1989. Each of these studies is consistent with the hypothesis that recruitment is related not to the absolute amount of substrate available, but rather to the amount of suitable substrate not already heavily populated by adult mussels.

Recruitment patterns in Lake St. Clair and some other European systems are also consistent with the intraspecific predation hypothesis. Laval settlement in Lake St. Clair was highest near adults in 1988 when adult densities were low but was most pronounced at population peripheries in 1989, by which time settled mussel densities had increased nearly 1000-fold (Hebert et al. 1991). Hebert and co-workers speculated that these patterns may have resulted from a chemical factor released by adults which triggered larval settlement when adults were present at low density, but which deterred settlement when adults were abundant. Chemical factors induce larval settling in many benthic invertebrates (see review by Burke 1986), but a dual facilitation–inhibition role is difficult to envisage.

Lewandowski (1982) reported that submerged Characeae (stoneworts) provided the best substrate for settlement of *Dreissena* larvae, followed in order by *Dreissena* colonies, stones, sand, and mud. Lewandowski (1982) suggested that these patterns may have resulted from substrate-specific settlement by postveligers. However, they are also consistent with the hypothesis of substrate-specific mortality following nonpreferential settlement. Postsettlement mortality agents and intensities could be substrate specific; for example, postveligers settling on sand or mud may lack sufficient structural support upon which to secure byssal threads or they may experience difficulty in feeding, while those settling on established colonies containing large size-classes may be eaten (Mikheyev 1967; Shevtsova et al. 1986) (Fig. 3 and 5). Lewandowski (1982) also described pronounced differences in age-class strengths among mussel populations residing on plants and in benthic colonies in Lake Rumian, Poland; plant-dwelling mussel distributions were highly skewed toward young (small) age-classes whereas colony-dwelling *Dreissena* were more equitably distributed among young and old age-classes. While these patterns may have resulted from preferential settling of larvae on plants and mortality of older plants and older mussels thereupon (Lewandowski 1982), they are also consistent with the hypothesis of differential mortality of larvae settling on plants and established *Dreissena* colonies. Clear differentiation between preferential settling by, and substrate-specific mortality of, postveligers will require carefully designed and executed field experiments. To date, only Sprung (1989) has observed high veliger mortality rates (99%) in the absence of predation. The only lake (Majcz Wielki, Poland) of which we are aware that clearly exhibited a total larval mortality rate lower than 99% is notable in that recruitment occurred primarily on "safe" settling substrates provided by plants (Lewandowski 1982). André and Rosenberg (1991) reported that settlement success of marine bivalve larvae was inversely related to the density of adults and reasoned that predation by adults may limit the recruitment of new individuals.

Dreissena Population Growth

Our noncannibalistic model clearly demonstrates that settled mussel densities observed in western Lake Erie during 1990 could not have been achieved if mortality of veligers to the settled subadult stage totalled 99%, even if optimistic reproductive schedules (Walz 1978b) and veliger inocula are employed (Table 3). Large increases in the fecundity of large mussels had little impact on time-to-current-density because these individuals represent only a small fraction of the total population (Lewandowski 1982; Mackie 1990; Hebert et al. 1991).

The composite model is consistent with population growth patterns in western Lake Erie. Populations in the basin are projected to demonstrate unrestricted growth for only the first few years, after which recruitment declines in relation to the density of predatory mussels. The recruitment decline observed in the lake between 1989 and 1990 (Garton and Haag 1990) can also be explained if exploitative competition between veligers, other herbivorous zooplankton, and benthic adult *Dreissena* resulted in high veliger mortality. This possibility seems unlikely because small-bodied zooplankton, such as rotifers, have low-threshold food levels (the food concentration at which energy intake equals metabolic losses) and should be less affected by declines in ambient food levels than larger species (Stemberger and Gilbert 1987). Veligers are similar to many herbivorous rotifer species with respect to body size and the manner in which food is collected (ciliated velum versus ciliated corona) and may have similar food requirements. Any increase in veliger mortality associated with low food levels should be preceded or accompanied by a decline in population densities of large herbivorous zooplankton.

It can be argued that current mussel densities in the western basin have resulted, in part, from migration of mussels from unsuitable substrates to adjacent suitable ones. While this possibility exists, on at least a very local scale, mortality to the settled subadult stage would still have to be less than 99% because mussel migration, and higher population densities associated with it, is incorporated into mortality calculations for European lakes (Stańczykowska 1977; Lewandowski 1982; Sprung 1989).

Our models utilized current western basin *Dreissena* population size structure to determine the mortality between age-class compartments. While we have not defined the mechanisms responsible for intercompartment mortality, intraspecific competition is a likely possibility. Hebert et al. (1991) noted that mussel growth was least pronounced in areas where sessile populations were in highest density. Intraspecific competition for food could skew population age structures toward small size-classes because large mussels have higher mass-specific metabolic demands and are more susceptible to food shortages than are smaller individuals (Walz 1978c).

In conclusion, our study demonstrates the predatory capability of adult *Dreissena* on Great Lakes rotifers and *Dreissena* larvae. Potential predation rates on rotifers exceed those of other invertebrate Great Lakes predators. Our models of mussel population growth in the western basin of Lake Erie indicate that mortality of larvae to the settled subadult phase could not have been as intense as previously described for European systems. We propose that larval mortality was initially substantially lower than 99% due to a lack of predatory adult mussels.

Acknowledgements

We thank E. Jin, D. Culver, C. Ramcharan, and D. Padilla for comments on the manuscript, E. Leitch and J. Gilbert for providing *Dreissena* and *Cryptomonas* cultures, and C. Ramcharan and J. Ackerman for providing us with relevant papers. Our study has been supported financially by a postdoctoral fellowship to H.J.M. and an operating grant to W.G.S. from the Natural Sciences and Engineering Research Council of Canada, by a Renewable Resources grant to W.G.S. and H.J.M. from the Ontario Ministry of Natural Resources, and by the Great Lakes University Research Fund from Environment Canada.

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Appendix 1.

A schematic representation of a STELLA™ (High Performance Systems, Inc. 1988) composite model for predicting *Dreissena* population growth in western Lake Erie is shown in Fig. A.1. *Dreissena* populations consist of veliger, adult 1, adult 2, and adult 3 subpopulations. Total veliger production is determined as $\sum_{i=1}^{3} \text{fecundity}_i \times (0.5 \times \text{adult}_i \text{ density})$ where i is age-class. Fecundities were initially set at 10^4, 2×10^4, and 3×10^4 eggs·female^{-1} for adult age-classes 1, 2, and 3, respectively. The model assumes an even sex ratio and that all eggs produced are viable and fertilized. Adult 1 age-class recruitment is determined as the product of the number of veligers available to settle (veligers), the environmental survival rate (environmental mortality), and predation survival rates (predation mortality). Composite model larval survival functions in relation to 2- and 3-yr-old mussel densities are shown below the model. Adult 1 age-class recruitment for the noncannibalistic model is identical except that it lacks predation mortality functions. In both models, 30% of age 1 individuals survive to age 2 (70% mortality), and 18% of these individuals survive to age 3 (82% mortality) (Leach 1991). All mussels live a maximum of 3 yr. The inoculum consisted of 5×10^8 veligers (see Materials and Methods). Densities of each adult age-class were calculated based on uniform distributions over $3.34 \times 10^8 \cdot m^{-2}$ of habitable substrate in the lake's western basin (Hartman 1973). The models iterate once per year. Fecundity values and environmental mortality rates were varied to determine the model's sensitivity (see Table 3).

NOTE ADDED IN PROOF

Our models assume that the initial cohort of veligers introduced to Lake Erie in 1986 were age 1 adults in 1987 and contributed to the veliger subpopulation for the first time in 1988. However, *Dreissena* populations in Lake Erie appear capable of reproducing in their year of settlement (i.e. individuals entering the age 1 subpopulation during 1987 reproduce and contribute to the veliger subpopulation in 1987). Our results are fairly robust to this modelling alternative. Using the range of larval mortality and adult fecundity values described in Table 3, and the same initial veliger inoculum, mean *Dreissena* densities observed in western Lake Erie during 1990 are predicted to be achieved between 1992 and 1994 for variants of the noncannibalistic model and between 1989 and 1990 for variants of the composite model.

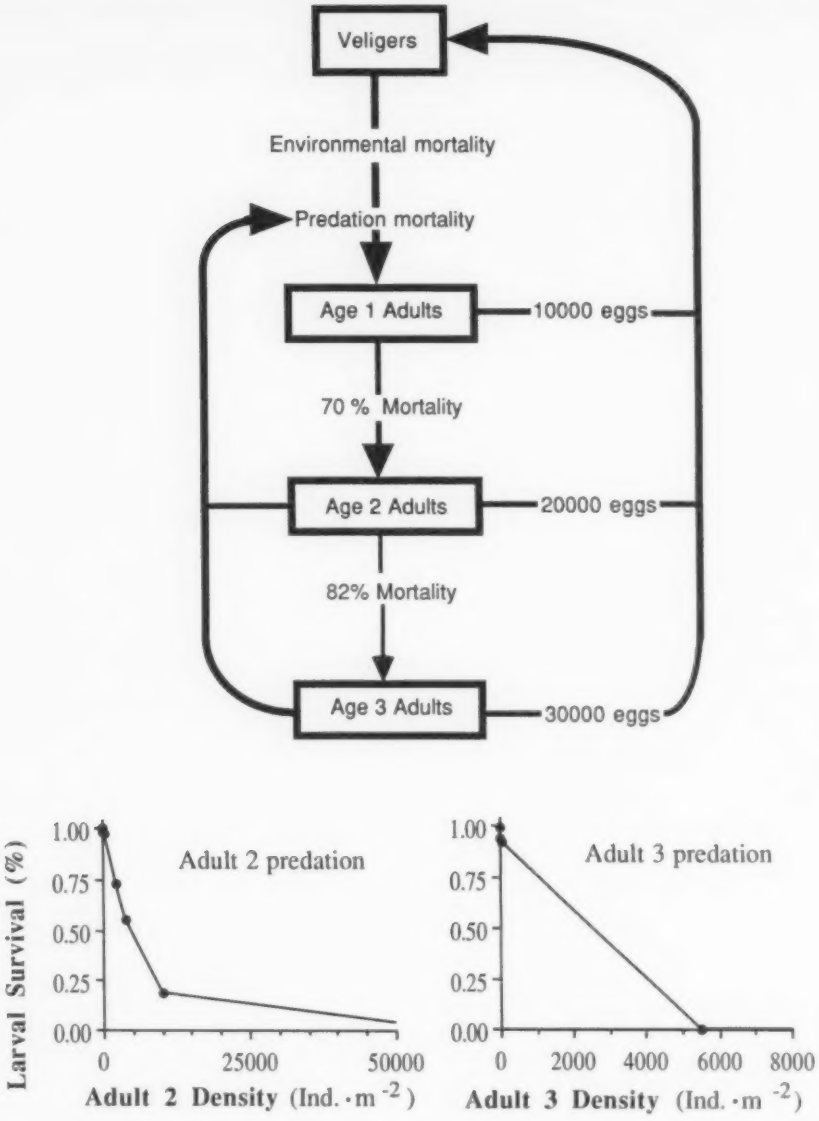

FIG. A.1. Schematic representation of a STELLA™ composite model.

Responses of Stream Periphyton and Benthic Insects to Increases in Dissolved Inorganic Phosphorus in a Mesocosm

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Mundie, J. H., K. S. Simpson, and C. J. Perrin. 1991. Responses of stream periphyton and benthic insects to increases in dissolved inorganic phosphorus in a mesocosm. Can. J. Fish. Aquat. Sci. 48: 2061–2072.

Responses of stream periphytic algae and benthic insects to increases in dissolved inorganic phosphorus (P) were assessed in a streamside mesocosm. Controls and treatments were colonized continuously in summer by biota from the stream. P was maintained in the treatments at 10 $\mu g \cdot L^{-1}$, ≥8 times the concentration in the controls. In the treatments the biomass of chlorophyll *a* reached 3.5 times that of the controls, accompanied by an increase in Cyanophyta relative to diatoms. No difference was detectable in the numbers of insects drifting from controls and treatments. Numbers of individuals emerging (>40 species) from the treatments over 7 wk were 2.2 times those from the controls. In both controls and treatments, 77% or more of emerging insects were Chironomidae. After week 7 the density of benthic insects in the treatments, determined without size selection, was 1.75 times that of the controls; size distributions in treatments and controls were similar. Addition of P, therefore, increased the food of insects and resulted in a doubling of their survival to emergence.

On a étudié les réactions des algues du périphyton et des insectes du benthos d'un ruisseau à l'augmentation de la teneur en phosphore (P) dissout, dans un mésocosme ripicole. Les parcelles témoins et traitées ont été colonisées continuellement durant l'été par des organismes du ruisseau. Dans les parcelles traitées, la concentration de P a été maintenue à 10 $\mu g \cdot L^{-1}$, ≥ 8 fois celle des témoins, et la biomasse de la chlorophylle *a* atteignait 3,5 fois celle des témoins, ce qui s'accompagnait d'une augmentation des cyanophycées par rapport aux diatomées. On n'a détecté aucune différence dans le nombre d'insectes dérivant des parcelles témoins et traitées. Sur 7 sem, il y a eu plus de 2,2 fois plus d'émergences d'insectes (> 40 espèces) dans les parcelles traitées que dans les témoins. Dans les deux cas, au moins 77 % des insectes émergeants étaient des chironomides. Après 7 sem, la densité des insectes benthoniques dans les parcelles traitées, établie sans sélection de la taille, était 1,75 fois celle des témoins; la distribution des tailles était la même dans les deux milieux. L'ajout de P a donc augmenté la nourriture pour les insectes, et donné lieu au doublement de leur survie jusqu'à l'émergence.

Received November 29, 1990
Accepted May 10, 1991
(JA829)

Reçu le 29 novembre 1990
Accepté le 10 mai 1991

Among the factors that can limit production of salmonid fishes in streams is the amount of invertebrate food available to them. Consequently, there have been recent attempts, some on a small scale and others on a field scale, to increase the food of salmonids either directly or indirectly. For example, shrimp (Euphausiacea) have been offered daily to juvenile coho salmon (*Oncorhynchus kisutch*) in a stream (Mason 1976); numbers of invertebrates have been increased via the heterotrophic foodpath by means of sugar (Warren et al. 1964) and cereal grain (Mundie et al. 1983; Perrin et al. 1987); inorganic nitrogen (N) and phosphorus (P) have been added to promote autotrophic production (Stockner and Shortreed 1978; Perrin et al. 1987). Each of these approaches shows promise as a means of increasing salmonid production; all warrant further research. The application of inorganic nutrients to a stream, for example, has demonstrated that rates of accrual of chlorophyll *a* can be increased by an order of magnitude, with diatoms (a suitable food for stream insects) predominating (Perrin et al. 1987). Consequently, comparisons of invertebrates in treated and control sites have shown three- to fivefold increases in biomass of standing stock in treated areas (Johnston et al. 1990). Details, however, of the kinds of invertebrates that responded to treatment and of the magnitude and timing of their responses are lacking, and there is need for more information.

This study was undertaken to clarify, in detail, the responses of stream insects, in the absence of fish, to increases in algae. Specifically, it asks: can a response from a natural assemblage of stream insects be demonstrated following an increase in production of their autotrophic food? If so, what insects respond and what are the magnitude and timing of their response? To answer these questions, changes in standing stock, drifting invertebrates, and emerging insects were examined.

Methods

Salmonids feed on a wide range of invertebrates; it was therefore realistic to use a multispecies test that incorporated many of the species of a natural stream. Interaction between two trophic levels (algae and invertebrates) was also involved. A mesocosmic approach therefore was taken. A mesocosm is an ecosystem simplification that retains a high degree of realism, yet reduces variability or isolates a mechanism; it gives repl-

icability on a manageable scale, and predictions, with respect to the isolated mechanism, valid for the natural system. Realistic features incorporated into the mesocosm were (1) natural species, (2) immigration and emigration of invertebrates, (3) colonization to establish a dynamic equilibrium between trophic levels and among functional groups, and (4) emergence of insects. The intention was to exclude fish, so that cropping of secondary production would not occur (occasional salmonid fry and one coastrange sculpin (*Cottus aleuticus*) did enter the troughs and were removed). Sampling extended over 7 wk on the assumption that responses from zoobenthos should be apparent in that time.

The experiment was carried out at Carnation Creek, a small, weakly acidic stream, low in nutrients, on the west side of Vancouver Island, British Columbia, at a site where the stream gradient was 8%. The stream has an annual maximum discharge of about 64 $mg^3 \cdot s^{-1}$ and a minimum discharge of 0.03 $mg^3 \cdot s^{-1}$. Further descriptive details are given in Scrivener and Brownlee (1989).

Experimental Apparatus

The experimental apparatus consisted of 12 parallel troughs placed on a wooden platform on the stream bank (Fig. 1a). Six control troughs (odd numbers) alternated with six treatment troughs (even numbers). This systematic arrangement was chosen to facilitate collection of material and labelling of vials and thereby reduce the likelihood of gross error in field sampling. It was assumed that the sequence could not correspond to any possible pattern in uncontrolled variation (Cox (1958) gave a discussion of the pros and cons of such systematic designs). Each trough contained gravel that was colonized by invertebrates entering with water drawn continuously by gravity feed from the creek. The water intake was a box-shaped cage of cord netting, which excluded debris, placed in the upstream end of a pool (Fig. 1b). Two polyethylene pipes (inner diameter 5 cm) conveyed water from the cage to the apparatus on the bank; they were thick walled to withstand possible interference from black bears (*Ursus americanus*). The pipes ran 100 m down the creek (vertical distance 2 m) to each end of a head tank on the bank (Fig. 1a).

On entering each end of the head tank, the water passed into a manifold (Fig. 1d) running the length of the tank between the inlets. The manifold served to control the flow and to distribute suspended materials evenly throughout the tank before they passed to the troughs. The manifold consisted of a polyvinyl chloride (PVC) pipe threaded into flanges at both ends, fitting snugly inside another PVC pipe which was not threaded, and was cut into three equal lengths. Both outer and inner pipes had a longitudinal slot cut in them (Fig. 1e) so that the amount of water passing, and its mixing in the head tank, could be controlled by rotating the slots in the three sections of the outer pipe relative to the slot in the inner pipe.

Water passed from the head tank to each trough via 12 PVC standpipes (Fig. 1f). These could be tilted slightly on their threaded elbows so that flows could be adjusted. Flexible nylon tubing conveyed the water from the standpipes to the troughs. A small excess of water was maintained by adjusting the standpipes, and this overflowed from the back of the head tank which was slightly reduced in height to allow this (Fig. 1f).

The troughs were of PVC, 122 cm long and 20 cm wide. Each was filled with gravel (diameter 13–20 mm) to a depth of

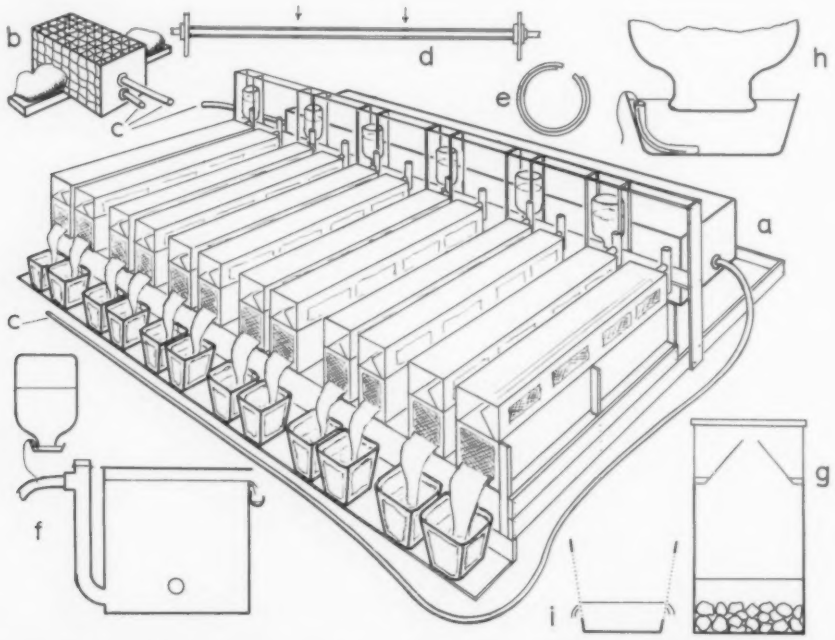

FIG. 1. Experimental apparatus: a, general view of troughs, emergence traps, buckets, bottles, and head tank; b, inlet cage; c, pipes for conveying water to head tank; d, manifold for distributing water in the head tank (arrows indicate cuts in outer pipe); e, cross-section of manifold; f, section of head tank, standpipe, bottle, and microbore tubing; g, cross-section of trough with gravel, and emergence traps; h, mouth of bottle, and dish for dispensing nutrients; i, section of bucket with screen walls.

5 cm. To maximize the holding capacity for insects, no sand was added. Water covered the gravel to a depth of 4 cm. Water left each trough by passing over a sill that determined water depth. To prevent adult insects from escaping, the downstream end of each trough above the outflowing water was covered with Nitex cloth (350 μm) secured with Velcro adhesive cloth.

Water leaving each trough dropped into a plastic bucket (Fig. 1i), square in cross-section and with sides having large rectangular cutouts covered with Nitex cloth (mesh 350 μm). The buckets retained drifting invertebrates.

Flows from the cage in the stream, through the pipes to the head tank, were started by drawing water into the pipes with a pump attached to the downstream end. Discharge was adjusted in each trough to 0.28 $L \cdot s^{-1}$. Surface velocity within the troughs averaged 5.3 $cm \cdot s^{-1}$. With this velocity, emerging insects were caught above the troughs before being carried out.

An insect emergence trap, of Plexiglas, covered each trough. The top was a removable lid. Inside each trap were longitudinal panels, sealed to a narrow ledge at their base and converging to within 2 cm of each other (Fig. 1g). Preservative (2% formalin plus wetting agent) was poured into each side of a trap and lay on the ledge. To allow passage of air, so that insects did not adhere to the wet walls and lid, the sides of the trays had cutouts covered with 350-μm Nitex cloth (Fig. 1a). Emergence traps were emptied weekly.

A recording thermometer was placed in one trough, and an electric fence was installed around the experimental facility to deter black bears from interfering.

Before nutrients were introduced on August 5 to the treatment troughs, flows passed through all troughs for 4 wk to allow colonization of the gravel by invertebrates from the stream. At the end of this period, thin sheets of Styrofoam (15 × 15 cm) attached to Plexiglas were placed on the gravel at the downstream end of all troughs to provide substrates for periphytic algae. Discs of Styrofoam with adhering periphyton were removed weekly by pushing the mouth of a light-tight plastic vial (inner diameter 31 mm) into the Styrofoam, any strands of algae that had clearly drifted on to the Styrofoam being excluded from the samples. The vials with the enclosed disc were capped and kept in a freezer at −15°C until analysis within 3 wk. These samples were used to determine periphyton accrual in response to treatment. On the final sampling date (September 20), an additional disc was extracted from each trough for taxonomic examination, the samples being preserved in Lugol's solution.

Nutrient Addition, Sampling, and Maintenance

From earlier nutrient analysis of Carnation Creek (Stockner and Shortreed 1978), there was evidence that the natural amounts of both P and N were imposing limits to autotrophic production, as the greatest increases in algal standing stock occurred following additions of both nutrients. Accordingly, concentrated solutions of ammonium nitrate (NH_4NO_3) and potassium dihydrogen phosphate (KH_2PO_4) combined were supplied to six treatment troughs. The solutions were delivered from narrow-necked glass bottles (fig. 1a, and 1f). The bottles were inverted into small dishes so that the solution flowed into the dish at a level maintained by atmospheric pressure, and a constant head resulted. Microbore tubing (inner diameter 0.58 mm, Teflon) siphoned the solution from the dishes to the nylon tubes that conveyed the water from the standpipes to the troughs (Fig. 1h). Turbulence at the head of each trough ensured mixing of the nutrient solutions with the incoming water. The rate of delivery of nutrients was adjusted by altering the length of the microbore tubing or the difference in height between its ends. Nutrient concentrations in the reservoir bottles were set to achieve final concentrations of 10 $\mu gP \cdot L^{-1}$ and 100 $\mu gN \cdot L^{-1}$ in the treatment troughs. In earlier studies of nutrient-deficient streams (Stockner and Shortreed 1978; Peterson et al. 1983; Perrin et al. 1987), these concentrations had resulted in order-of-magnitude increases in accrual of periphyton, and they are higher than levels known to saturate N- and P-limited growth of periphyton (Bothwell 1988).

TABLE 1. Mean inorganic P and N concentrations ($\mu g \cdot L^{-1}$) in control (C) and treatment (T) troughs by date.

	SRP		NH_4-N		NO_3-N		DIN	
Date	C	T	C	T	C	T	C	T
Aug. 9	0.3	5.6	2.9	37.6	104	153	107	191
Aug. 16	0.2	5.0	8.7	40.7	138	166	146	206
Aug. 23	1.5	9.8	6.4	41.5	108	138	115	179
Aug. 30	1.5	10.3	7.2	43.7	84	118	92	162
Sept. 6	1.3	8.9	10.1	42.9	92	120	102	163
Sept. 13	0.9	12.7	7.6	49.4	115	160	123	209
Sept. 20	0.7	6.4	7.9	37.2	178	211	186	248

One set of water samples for analysis of total dissolved P, dissolved reactive P, nitrate, and ammonium was collected at the outflow of each trough before nutrient introduction started. Samples were then taken weekly. They were filtered in the field through a 0.45-μm membrane filter and analyzed within 48 h.

Although sampling was weekly, daily inspection was necessary. The fine-bore Teflon tubing could clog on minute particles of debris. This was largely overcome by covering the dishes with fine gauze. The buckets that collected the drifting materials could clog, after several days, with filamentous algae. This was remedied by removing most of the accumulated contents in midweek and preserving them.

At the conclusion of the experiment the sediments and benthic invertebrates of each trough were collected with a Nitex net of mesh size 50 μm. Size selection of insects was therefore avoided (Mundie et al. 1983). The flows in a trough were stopped and the net was placed at the outlet. The gravel in the trough was agitated with a small garden fork and the loosened material was washed repeatedly, with water from a bucket, into the net. This process was continued for a standard time of 6 min. When the net blocked with sediment, it was replaced with a clean one.

Laboratory Analysis

Phosphate and nitrate concentrations were determined by the methods outlined in Stephens and Brandstaetter (1983). Chlorophyll was determined by the procedure of Holm-Hansen et al. (1965). Algae from Styrofoam discs were settled in Utermöhl chambers (Perrin et al. 1987) and the percentage composition of genera was determined by counts of cells at 500 × magnification along 10 transects. Counts were restricted to genera that occupied ≥10% of the sample.

Clear differences between the concentrations of soluble reactive phosphorus (SRP) and dissolved inorganic nitrogen (DIN) in control and treatment troughs confirmed the magnitude of nutrient addition (Table 1). SRP levels in the control troughs were extremely low throughout the experiment (0.2–1.5 $\mu g \cdot L^{-1}$). Phosphate additions increased SRP levels by 20-

fold in the first 2 wk, but the difference declined to about eightfold thereafter. With the exception of the first 2 wk, SRP levels were about 10 $\mu g \cdot L^{-1}$ during the nutrient addition. DIN concentrations generally exceeded 100 $\mu g \cdot L^{-1}$ regardless of the ammonium nitrate addition. Ammonia was only 5% of DIN (<10 $\mu g \cdot L^{-1}$) in the control troughs, but increased to about 20% of DIN (40 $\mu g \cdot L^{-1}$) due to the nutrient addition. The combined addition of ammonium and nitrate increased DIN levels to a range from 162 to 248 $\mu g \cdot L^{-1}$ (Table 1). Since natural temporal variations in DIN concentration were not predictable, additions of N were continued, but in retrospect these proved to be unnecessary because control levels were near the target for the N treatment.

One-way ANOVA's of SRP and DIN concentrations (controls and treatments run separately, with dates combined) showed no trough effect for either nutrient ($P < 0.05$). Thus the treatment troughs all received similar levels of nutrient addition, and background concentrations were consistent across the water supply.

Collections of drifting macroinvertebrates were sorted directly, in small amounts at a time, under a stereomicroscope, since the presence of filamentous algae prohibited satisfactory subdivision. Analysis of all collections proved to be prohibitively laborious (approximately 12 h per sample), so only two of the seven series were examined. Zoobenthos was counted after settling the material collected from each trough into a "honeycomb" of 80 tubes (2.5 cm diameter, 9.5 cm high) standing in a bucket half full of water, after the method of Mundie et al. (1983). This made subsampling possible. It was not necessary to pour the sample, firstly, through an 8-mm sieve, for little coarse debris was present. Ten tubes were drawn at random for making counts under a stereomicroscope capable of 50× magnification. Body lengths of insects were measured approximately, under the stereomicroscope, with the specimen placed on a measuring scale marked in millimetres.

At the conclusion of the experiment, gut contents of miscellaneous ($n = 90$) chironomid, mayfly, stonefly, and caddis larvae collected from the control and treatment troughs were examined to determine whether a change in proportions of diatoms relative to detrital particles had taken place. The analysis was confined, however, to preserved insects collected at midday.

Results

Benthic Algal Biomass and Composition, and Diet of Insects

During all 7 wk of the experiment, benthic algal biomass was significantly greater in the troughs receiving additional nutrients than in the control troughs (Fig. 2). Chlorophyll *a* concentrations increased exponentially for the first 21 d and thereafter were sustained at a mean of 16.2 $mg \cdot m^{-2}$ in the control troughs and 58.5 $mg \cdot m^{-2}$ in the treatment troughs. Peak biomass was 19.2 and 67.7 $mg \cdot m^{-2}$ in the controls and treatments, respectively.

At the end of 7 wk, diatoms (Bacillariophyceae) predominated in both control and treatment troughs (Table 2), their percentage abundance being 80.5 and 70.0, respectively. The chlorophytes, represented by *Oedogonium* sp. and *Cosmarium* sp., amounted to less than 10% of all numbers. The cyanophytes consisted mainly of *Oscillatoria* sp. In the control troughs, they made up 10%, but this proportion increased to 23% with treatment. This shift was the most notable

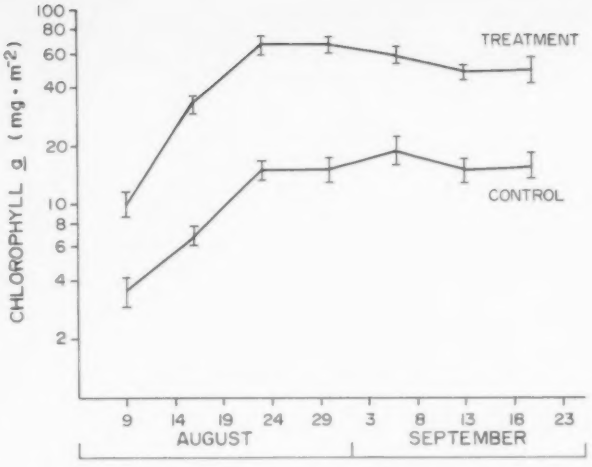

FIG. 2. Mean algal biomass (± 1 SE) shown as chlorophyll *a* concentrations on Styrofoam substrates in control and treatment troughs during 7 wk of nutrient addition.

TABLE 2. Proportions (%), as counts of cells, of common periphytic algae on Styrofoam substrates in control and treatment troughs on September 20.

	Control	Treatment
1. Phylum or class		
Bacillariophyceae	80.5	70.0
Chlorophyta	9.3	6.5
Cyanophyceae	10.2	23.5
2. Species composition		
Bacillariophyceae		
Achnanthes sp.	30.5	33.0
Tabellaria spp.	22.5	15.8
Hannaea arcus	18.3	10.0
Synedra sp.	10.0	10.8
Cocconeis placentula	6.0	14.2
Cymbella sp.	3.3	11.2
Chlorophyta		
Oedogonium sp.	49.2	70.0
Cosmarium sp.	46.7	28.3
Cyanophyceae		
Oscillatoria sp.	98.0	98.3

change found. *Achnanthes* sp. was the commonest diatom, followed by species of *Tabellaria*. *Hannaea arcus*, a species typical of mountain streams, was relatively more abundant in the control troughs whereas the pennate alga *Cocconeis placentula* and *Cymbella* sp. were relatively more common in the treatment troughs.

Analysis of gut contents of larvae did not show diatoms to be more abundant than detrital particles, nor were they more abundant in the treatment samples than in the controls. Moreover, the frequency of occurrence of filamentous algae in the samples as a whole was unexpectedly high (88%). The samples were limited, however, to one time of day and their sizes were small.

Drifting Invertebrates

Two series of invertebrates that accumulated over 7 d in the buckets at the outflows of the 12 troughs were analyzed

TABLE 3. Weekly accumulations (means) of drifting invertebrates collected from the buckets.

	Week ending August 30		Week ending September 6	
	Controls	Treatments	Controls	Treatments
Chironomid larvae	232	245	232	285
Ephemeropteran larvae	80	77	171	142
Trichopteran larvae	41	29	39	29
Plecopteran larvae	0.3	0.1	0.8	1.6
Chironomid adults	6.6	23	6.6	20
Ephemeropteran subimagoes	3.6	30	3.1	15
Simuliid larvae	1.6	2.5	0.8	1
Dixid larvae	0	0	0.3	1
Gordius sp.	1.1	1.3	2.8	0.6

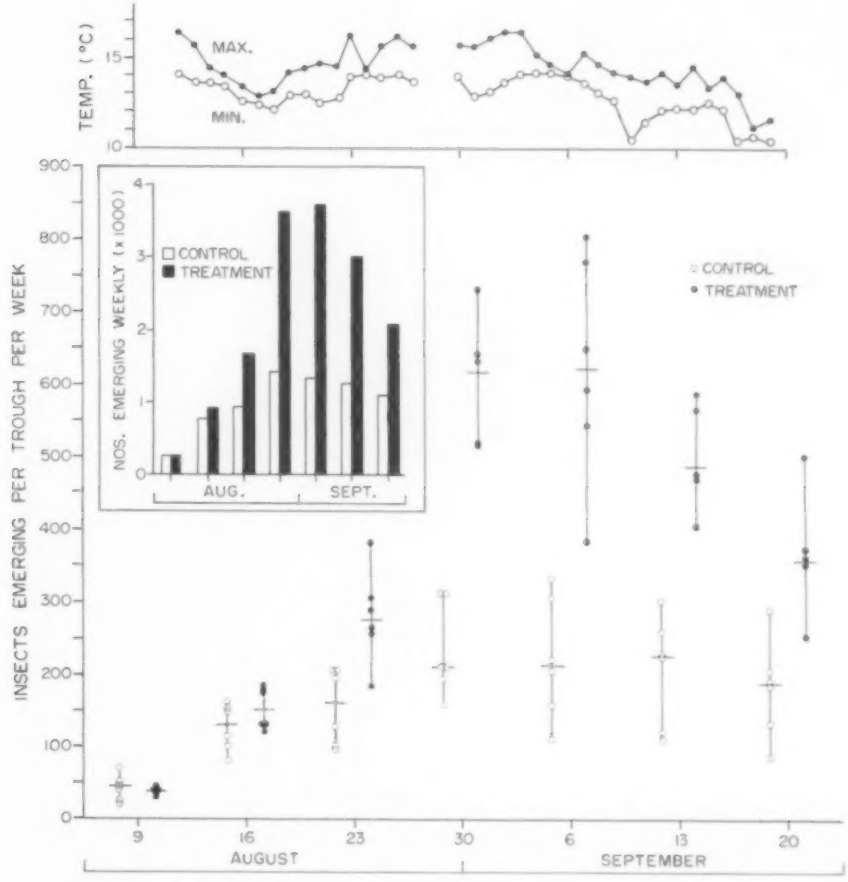

FIG. 3. Counts and medians of insects emerging per trough per week. Each point represents a count from a trough. Inset: total numbers emerging weekly from all control and all treatment troughs.

(Table 3). Small numbers of chironomid and ephemeropteran larvae that were found, that were capable of passing through the 350-μm mesh of the Nitex screen on the sides of the buckets, were omitted from the counts. The numbers of larval chironomids, mayflies, and caddisflies drifting from the control and treatment troughs did not differ (Wilcoxon's sum of ranks test, $U = 18$–24, $P > 0.05$). The chironomid adults and ephemeropteran subimagoes were not the products of drift, but of emergence.

Emerging Insects

Addition of nutrients to the troughs started on August 5. On August 9 the cumulative week's emergence of insects (Table 4; Fig. 3) was similar in the control troughs (total 253 insects, 71% chironomids, and 28% ephemeropterans) and in the treatment troughs (total 224 insects, 67% chironomids, and 29% ephemeropterans). After 2 wk, treatment troughs produced greater numbers, and this difference was maintained for 4 more

TABLE 4. Numbers of emerged insects caught from control (C) and treatment (T) troughs on three occasions.

	August 9		September 6		September 20	
	C (6 troughs)	T (6 troughs)	C (2 troughs)	T (2 troughs)	C (2 troughs)	T (2 troughs)
Ephemeroptera						
Baetidae						
Baetis sp.	64	63	33	53	1	27
Leptophlebiidae						
Paraleptophlebia sp.	7	4	3	1	3	7
Plecoptera						
Leuctridae						
Despaxia sp.	1	1	1	3		
Chloroperlidae						
Alloperla sp.	1	0				
Trichoptera						
Polycentropodidae						
Polycentropus sp.			1	0		
Rhyacophilidae			1	1		
Hydroptilidae						
Agraylea sp.	2	2	3	6		
Lepidostomatidae						
Lepidostoma sp.			2	2	0	1
Diptera						
Chironomidae						
Boreochlus sp.			0	1		
Paramerina (?) sp.	60	36	0	9		
Thienemannimyia sp.	9	8				
Zavrelimyia sp.	4	9	37	37	36	40
Pagastia sp.					0	1
Brillia sp.	4	3	0	8	2	0
Cardiocladius sp.			1	0		
Corynoneura sp.	13	18	19	76	31	59
Cricotopus sp. *a*	5	5	9	65	11	6
Cricotopus sp. *b*	1	0	5	14	1	0
Cricotopus sp. *c*			0	11	3	2
Eukiefferiella sp. *a*	9	22	18	60	16	39
Eukiefferiella sp. *b*			11	0		
Heterotrissocladius sp.			3	0	0	1
Rheocricotopus sp.			0	3		
Orthocladius sp. *a*	3	4	0	5	7	0
Orthocladius sp. *b*	10	1	21	117	17	40
Orthocladius sp. *c*	8	5	10	0		
Orthocladius sp. *d*					4	1
Orthocladius sp. *e*			14	0	0	2
Orthocladius sp. *f*					0	1
Orthocladius sp. *g*			0	4	1	0
Synorthocladius sp.	2	0	4	488	0	172
Thienemanniella sp.	5	11	10	36	13	40
Micropsectra sp. *a*	33	22	18	57	10	5
Micropsectra sp. *b*			0	5	89	112
Microtendipes sp. *a*			76	83	25	13
Microtendipes sp. *b*			0	1		
Phaenopsectra sp.			0	1		
Polypedilum sp.	13	8	19	19	4	19
Cladotanytarsus sp.			42	143	4	3
Tanytarsus sp.			0	4	0	7
Simuliidae						
Simulium sp. *a*	0	2	18	15	8	9
Simulium sp. *b*			0	1	1	0

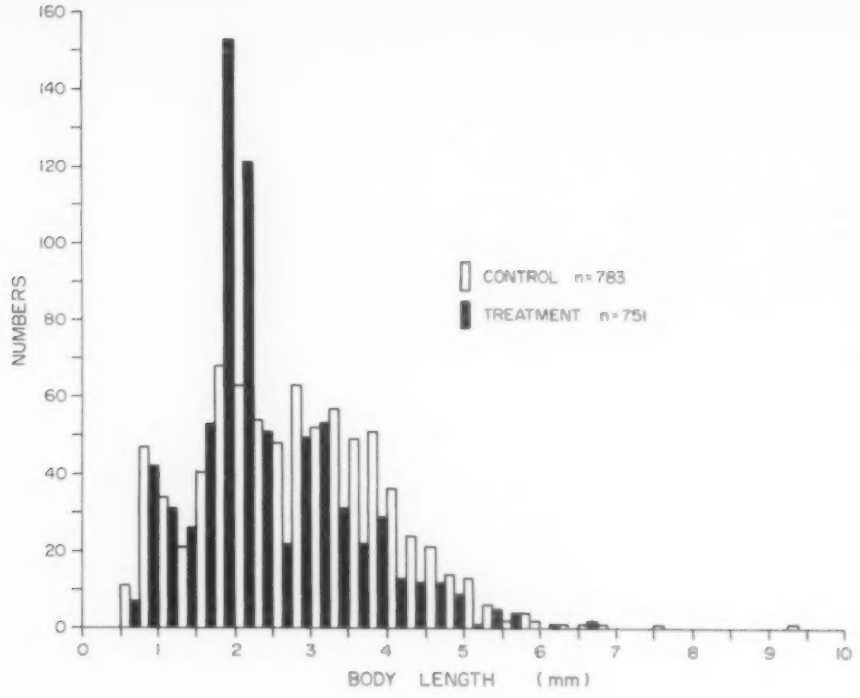

FIG. 4. Size distribution of emerged insects from controls and treatments on September 6.

TABLE 5. Totals over 7 wk of emerged adults of major groups from control (C) and treatment (T) troughs.

	C	T	Ratio
Chironomidae	5924	13516	1:2.28
Ephemeroptera	627	1326	1:2.11
Trichoptera	84	125	1:1.48
Plecoptera	34	46	1:1.35
Simuliidae	272	220	—

wk, when the experiment was concluded (Fig. 3). The difference in mean numbers between controls and treatments over 7 wk was 2.2-fold; the maximum difference occurred on the fifth week (September 6) and was 2.8-fold. Emergence from treatment troughs, but not from the controls, showed a marked decline during September. Throughout the 7 wk the proportions of chiromonids in both control troughs and treatment troughs increased steadily. On August 23, they were 77% in both; by September 20, they were 95% in the controls and 94% in the treatments.

To determine whether size differences occurred in the emerging insects as a result of treatment, the body lengths of chironomids, mayflies, and caddisflies collected on September 6 were measured (Fig. 4). Sizes were broadly similar in controls and treatments, except that in the treatments there were conspicuously more chironomids in the range 1.76–2.25 mm, and fewer adults greater than 2.50 mm. To test for significance of the difference, the size categories were reduced to 21, and the summed χ^2 values were calculated for the differences between observed and expected frequencies of the two groups. The difference was significant ($\chi^2 = 115.4$, df = 20, $P = 0.000$). The taxonomic data of Table 4 support the small size peaks of Fig. 4. This indicates that enrichment favoured the smallest chironomid species, e.g. *Corynoneura*, *Thienemanniella*, *Synorthocladius*, and *Cladotanytarsus*.

Table 5 shows the total numbers of adults of major groups that emerged from the control and treatment troughs. Analysis of the weekly catches of adults on three occasions gave 40 species, with chironomids predominating (Table 4).

Zoobenthos

On September 20 the head tank was sampled to determine whether it supported a significant and distinctive fauna that might have influenced the colonization of the troughs. A fine layer of detritus and sand was found. This contained a very sparse miscellaneous fauna of chironomid, mayfly, stonefly, and caddis larvae, along with numerous empty chironomid tubes. It was concluded that the fauna of the head tank had not had an influence on the contents of the troughs.

The mean settled volumes of sediments, with fauna, collected from controls and treatments on September 20 were 226 and 228 mL; the range for all samples was 173–239 mL. These values indicate an even distribution of material to the troughs. Counts of zoobenthos from this material after it was settled into tubes gave a median number of invertebrates in the treatment troughs of 1.72 times that of the control troughs (Fig. 5), the difference being significant (Wilcoxon's sum of ranks test, $U = 36$, $P \leq 0.005$). The 95% confidence limits of the difference between the medians were 1.37 and 2.02, based on the Mann–Whitney U test (Daniel 1978). The mean number in the treatments was 1.75 times that of the controls. The counts of the major taxonomic groups in the control and treatment troughs showed that the greatest response to nutrient addition was from the chironomids and Ephemeroptera (Table 6).

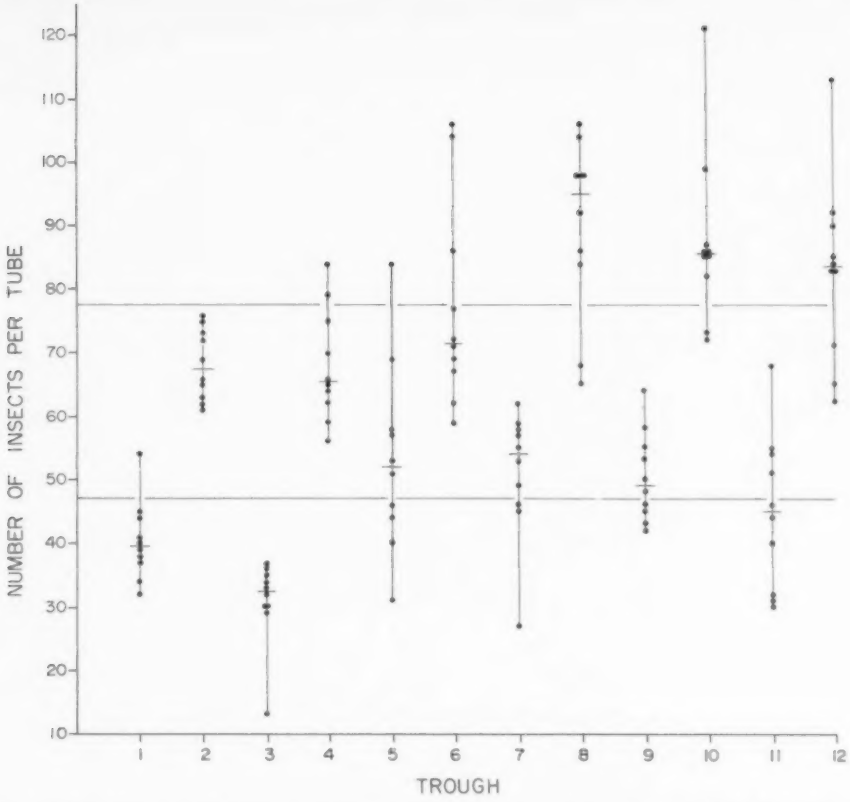

FIG. 5. Subsamples of total benthos collected from each trough on September 20. Each point represents the number of animals settled into a tube and counts were made of 10 tubes per trough. Odd numbers are control troughs and even numbers are treatment troughs. Vertical lines are ranges, short horizontal lines are medians, and long horizontal lines are overall medians for controls and treatments.

TABLE 6. Counts of settled benthic invertebrates collected on September 20 from control (C) and treatment (T) troughs.

	C	T	C:T	*P*
All items	2711	4738	1:1.74	<0.001
Chironomidae (larvae)	2045	3705	1:1.81	<0.001
Ephemeroptera	301	521	1:1.73	<0.001
Trichoptera	244	353	1:1.44	<0.001
Plecoptera	81	91	1:1.12	ns

The percentage composition (n = 2711) in the control troughs was Chironomidae (larvae and pupae) 77, Ephemeroptera 11 (mainly *Paraleptophlebia* and *Ameletus*), Trichoptera 9 (mainly *Agraylea, Micrasema*, and *Hydropsyche*), Plecoptera 2 (*Alloperla* and an unidentified perlodid species), and Ceratopogonidae, Tipulidae, Annelida, and others each less than 1%. The corresponding percentages (n = 4738) in the treatment troughs were 79, 11, 7, and 1, with the remaining items again each less than 1%. No notable change in proportions, with treatment, therefore, was apparent. The increases in yield over 7 wk, therefore, of some groups as a result of enrichment were not reflected in a shift in proportions of these groups in the benthos at the end of this period.

The size distributions of the control and treatment benthos were compared to see whether there was a change in response to treatment. The distributions of the more common items were similar (Fig. 6), with a modal body length for chironomid larvae in the category 1.0–1.25 mm in both controls and treatments.

The counts from 10 tubes amounted to 8.46% of the total sample (allowing for insects that settled between tubes). The total number of animals in the control troughs was therefore 5319, or 22 120·m^{-2}; the mean number in the treatment troughs was 9337, or 38 840·m^{-2}.

Discussion

The results show that insects respond to enrichment with a 2.2-fold increase in numbers of emerging insects over 7 wk and a 1.75-fold increase in density of benthos at the end of that period. The groups which show greatest response are Chironomidae and Ephemeroptera. Of the chiromonids the greatest response is shown by very small species. The timing of the response is best seen from the emergence data. The addition of food is interpreted as resulting in lower larval mortality, so the ends of the survival curves of species are slightly elevated. The proportion, however, of benthic insects that is nearing emergence at any time is a small part of the total (only 1.8% of the benthos collected from the control troughs and 2.4% from the treatment troughs were pupae). Consequently, changes in num-

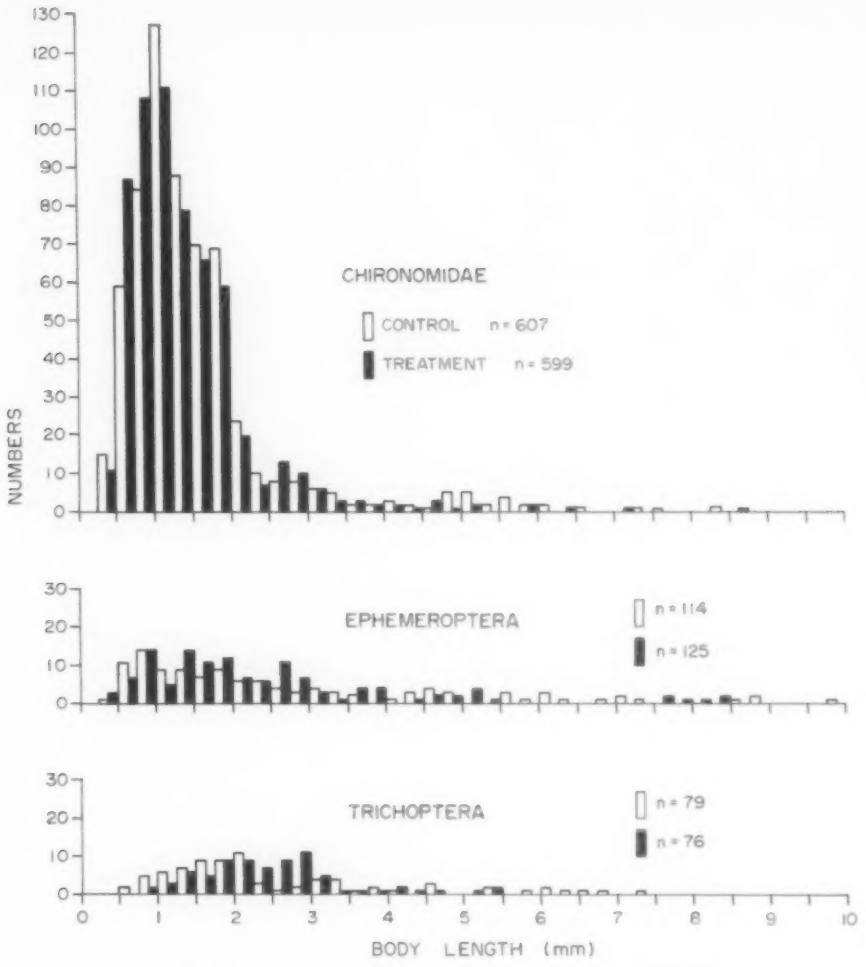

FIG. 6. Size distribution of benthic insects in control and treatment troughs.

bers of total benthos in response to enrichment of a stream are not likely to be detectable in the first month of treatment, but changes in weekly catches of emerging insects should be detectable. Changes in benthic density and biomass should be most apparent after 3 or 4 mo of nutrient additions to a stream, when second and third generations of insects respond to sustained high levels of available food.

Chemistry, Periphyton, and Diets

The mesocosm experiment gave the same result as that obtained by Stockner and Shortreed (1978), in that the accrual of periphytic algae in Carnation Creek was greater with the addition of inorganic P. Whereas biomass of chlorophyll *a* increased ninefold in the earlier work, it increased only 3.5-fold in this study. In another study, the chlorophyll *a* of diatom communities on Styrofoam substrates reached 100–200 $mg \cdot m^{-2}$ with N and P additions (Bothwell 1989). The discrepancy in the magnitude of these responses may be due to differing trough environments. The velocity adopted by Stockner and Shortreed (1978) was 40 $cm \cdot s^{-1}$, that of Bothwell (1985, 1988, 1989) was 50 $cm \cdot s^{-1}$, and that of the present study was 5.3 $cm \cdot s^{-1}$. In general, periphyton growth rates tend to increase with increasing velocity and consequent improved turbulent diffusion.

In spite of the low velocity in the experimental troughs at Carnation Creek the community which developed consisted mainly of diatoms. The filamentous algae (*Ulothrix*, *Oedogonium*, and *Zygnema*) which accumulated copiously in the buckets were transported there mainly from the creek, where they occurred most noticeably, not in pools, but in shallow swift water.

Although autotrophic production in Carnation Creek was found to be limited by both N and P in the study by Stockner and Shortreed (1978), this condition did not hold in 1988. Ambient nitrate concentrations had increased from an average of 70 $\mu g \cdot L^{-1}$ in 1976 to more than 100 $\mu g \cdot L^{-1}$; the latter value was the intended target level for N addition in 1988. In 1988 at Carnation Creek the algal community was limited by P only.

Another factor contributing to the relatively low biomass response of the algae to treatment is herbivory by insects. It is noteworthy, however, that the insects did not crop the algae

down to the level of the control troughs. The reason why this was so remains obscure. By analogy with ungulates cropping grassland, the diatoms should be consumed until they become difficult to obtain. Some factor would seem to be setting limits to cropping rates. It cannot be assumed, however, that algal accrual and herbivory on Styrofoam correspond to accrual and herbivory on gravel (Aloi 1990). In attaining a twofold increase in survival the insects probably reduced the periphyton substantially. The ability of stream insects to reduce the biomass of primary producers has been observed in both laboratory and field studies (reviewed by Gregory 1983). Orthocladiinae by themselves can control the amounts and kinds of algae in large outdoor channels (Eichenberger and Schlatter 1978); populations of *Corynoneura* larvae have been found to have a mean grazing rate from April to September of 25.6 mg (dry wt) of periphyton·m^{-2}·d^{-1}, and this was sufficient to affect periphyton standing stock (Kesler 1981).

A taxonomic change that occurred with the treatment was an increase in proportions of blue-green algae. It is known that the N-fixing blue-green algae are weak competitors for available P; hence, at very low concentrations of P, their relative abundance is low. When P was increased the cyanophytes became more common; nevertheless the diatoms predominated.

The frequency of occurrence of filamentous algae in the diet of the larval insects is notable. In general, stream herbivores are considered to reject filamentous or blue-green algae (Gregory 1983), presumably mainly because of their thick cell walls. Nevertheless, filamentous algae can be significant food for a variety of freshwater invertebrates; e.g. the minute larvae of *Agraylea* are highly adapted for holding and biting the cell walls of filamentous algae so that they can puncture them and then suck out the cell contents (Nielsen 1948).

Although the objective of adding P to the water of Carnation Creek was to produce more diatoms for insects, the addition of P would also promote heterotrophic production from which insects would benefit. An example of a coupling of algal and bacterial activity that benefits a stream chironomid is found in the diet of *Orthocladius rivulorum* Kieffer. This midge feeds on the diatom *Hannaea* that becomes attached to its tube of silk. Bacterial colonies flourish on the diatoms and respond to additions of P (Hershey et al. 1988). Another heterotrophic pathway that is influenced by the addition of P is leaf decomposition by microbial activity (Elwood et al. 1981). This decomposition will also promote secondary production.

It would be of value to be able to compare insect emergence rates during inorganic enrichment with those during organic enrichments. Changes in numbers and weights of zoobenthos, as established by Mundie et al. (1983) for organic additions, should be supplemented with emergence data. (The flows given on p. 1703 of the 1983 paper are erroneous. Discharge in each trough is given as 1 L·min^{-1}; this should read 1 L·s^{-1}.)

Drifting Invertebrates

The average count of drifting insects per day per trough, for the two series examined, was 58. This amounted to 1.09% of the benthos of a control trough and 0.62% of the benthos of a treatment trough. Most of the drift, however, collected in the buckets was probably derived from the creek, not from the troughs, and was influenced by natural discharge. Simuliid larvae were not likely to have been produced in the troughs themselves, on account of the low water velocity, but to have entered from the creek, or more likely from the intake pipes which afforded a favourable habitat for them. Dixid larvae (Diptera, Dixidae) and the horsehair worm *Gordius* sp. (Nematomorpha), some of which attained a length of 17 cm, presumably also entered the troughs from the creek. A single sample (from the bucket below trough 6), containing 7 d of accumulation ending on August 16, yielded 999 chironomid larvae and 114 simuliid larvae. These high numbers seemed to have resulted from catastrophic drift (Waters 1965) following unusually high rainfall (27 mm) on August 15. The mesocosm experiment did not therefore detect whether enrichment influenced drift rates of insects.

Emerged Insects

Emerged insects, although traditionally a neglected parameter in evaluation of changes in streams, proved to be the most informative parameter of this study. They gave a measure of relative survival in the control and treatment troughs to the reproductive phase, they provided adults which could be identified within the limits of current knowledge, and they demonstrated the degree to which proportions of species changed under treatment. The reason for the decline in emerged insects in the treatment troughs during September is obscure. It may be related to the influence of grazing pressure on the algae (Table 2), for this can produce marked changes in algal composition and abundance (DeNicola et al. 1990), but this is conjectural.

Zoobenthos

It is assumed, and it is a prerequisite of the experiment, that as a result of even dispersal of suspended materials from the head tank, approximately equal numbers of invertebrates entered all troughs during the experiment. No difference could be detected between the numbers leaving control troughs and those leaving treatment troughs. The high density of benthos found in the treatment troughs on September 20 is therefore attributable to higher survival of insects that entered and remained there. This higher survival is the result of greater amounts of available food. From the emergence data, it can be inferred that density of benthos in the treatment troughs exceeded that of the controls from at least the third week of August.

Water quality in the control troughs, and temperature in all troughs, corresponded to that of the creek. Similarly, although light was not measured, there was no reason to suspect that light reaching the troughs deviated significantly from that reaching much of the creek. The main questions concerning zoobenthos relate to the influence of substrate and water velocity in the troughs, to the densities attained, and to the species showing most response.

The factors of substrate (particle size and composition, silt, organic content, and stability) and water velocity are closely interrelated, so it is often not possible to separate their individual effects (reviewed by Minshall 1984). In natural streams, a fast current is associated with coarse substrate, some sand content, and low accumulations of organic matter. In the troughs, however, the conditions were slow current, fairly coarse substrate (approximately 13–20 mm diameter), absence of sand, large interstitial spaces, and, presumably, significant accumulations of organic matter. In spite of low water velocity, therefore, the rate of perfusion of water through the gravel would have been much higher than that of a natural riffle.

The genera *Micrasema*, *Hydropsyche*, *Paraleptophlebia*, *Ameletus*, *Eukiefferiella*, and *Tanytarus*, found in the troughs, are classified (Merritt and Cummins 1984) as belonging generally to lotic erosional habitats whereas *Corynoneura* and *Micropsectra* are typically lotic depositional forms. *Agraylea*, *Thienemanniella*, and *Cricotopus* are recorded from both erosional and depositional localities. It appears, therefore, that the fauna of the troughs was broadly a riffle fauna with the filter feeders greatly reduced. Filter feeders in Carnation Creek itself, however, are represented only by simuliids which make up <2% of the riffle zoobenthos (Tschaplinski 1987).

Uniform-sized gravel, when placed in streams and colonized by insects, tends to collect an assemblage of species similar to that of the natural environment but substantially different in relative or absolute abundance (Shaw and Minshall 1980; Pontasch and Cairns 1989). In the early phase of colonization, however, chironomids and baetids may predominate (N. H. Anderson, pers. comm.). A remarkable example of a chironomid rapidly colonizing clean gravel is afforded by *Orthocladius calvus* Pinder which can attain a density of 68 600 larvae·m^{-2} by oviposition (Ladle et al. 1985). This high density fell very substantially over 33 d.

Being uniform in particle size, the gravel in the troughs differed greatly from that of the creek. When insects colonize gravel of uniform size, higher densities build up on intermediate-sized gravel (approximately 25 mm diameter) than on smaller or larger gravel (Minshall and Minshall 1977; Rabeni and Minshall 1977), although biomass may be greater on smaller particles (Williams and Mundie 1978). This result is usually attributed to the greater capacity of intermediate gravel to retain particulate organic matter. Precise comparison, however, of the densities reached on intermediate gravel with those of natural riffles is elusive because of the difficulties of obtaining validly comparable samples. Nevertheless, evidence indicates that intermediate gravel supports more fauna than natural gravel (Shaw and Minshall 1980). Using a net of mesh 250 μm, Tschaplinski (1987) obtained a mean density of the riffle fauna in Carnation Creek, in September, of 9500 invertebrates·m^{-2}. The estimate of the fauna in the treatment troughs, obtained with a net of mesh 50 μm, was 38 840·m^{-2}. Although food was provided, the density reached was not high.

Moderate density of insects in the troughs was shown also by the emergence rate. The maximum emergence rate for chironomids was recorded from treatment trough 10 during August 31 to September 6. This produced 457 chironomids·m^{-2}·d^{-1}. An emergence rate, however, of 818 chironomids·m^{-2}·d^{-1} (mostly minute species, e.g. *Corynoneura* and *Thienemanniella*) has been recorded during August from riffle substrate and catches of 100 chironomids·$m^{-2}d^{-1}$ from pool substrates (Wartinbee 1976).

It is not clear what factors set a limit to the population densities in the troughs, but it is possible that substrate preferences are of less consequence than the amount of fine particulate organic matter available as food, or the perfusion rates that convey particles through the gravel. Other factors that influence densities of benthic invertebrates are pH (e.g. see Collier and Winterbourn 1987) and water velocities sufficient to dislodge and transport algae (Horner et al. 1990).

The difference in density of zoobenthos between the control and treatment troughs is assumed to be the result of the treatment having increased available food, the control troughs giving a measure of what the creek could produce on a standardized substrate without additional nutrients. This difference is therefore a measure of increased carrying capacity. If this is so, the availability of food per capita should have been the same in controls and treatments. Consequently, differences in growth rates of individuals in controls and treatments would not be expected, although sizes of particular species were not examined. This interpretation of food/density relationships is supported by the findings of Hawkins (1986) for ephemeropteran nymphs, i.e. increases in food support denser, but not faster growing, populations. Similarly, density-dependent growth of *Ameletus* (Siphlonuridae) nymphs feeding on diatoms in channels installed in a creek was noted by Hill and Knight (1987). Presumably, individuals that were in excess of carrying capacity in the Carnation Creek experiment would leave the troughs by drifting (Kohler 1985).

The response of the smallest chironomids to enrichment is particularly noteworthy because of their short life cycles. There is growing acceptance (Stites and Benke 1989) that the high development rates found under laboratory conditions by Mackey (1977) occur also under field conditions. Mackey obtained a larval development time of 8.7 d at 15°C for *Synorthocladius semivirens* (Kieffer), 5.0 d for *Corynoneura coronata* Edwards, and 10.0 d for *Cladotanytarus atridorsum* (Kieffer). *Thienemanniella majuscula* (Edwards) can attain five generations a year at temperate latitudes (Tokeshi 1986). These data indicate that the trophic significance of these insects has been underestimated. Because they constitute small particles with a high turnover rate, and because of their high numbers, very small chironomids appear to be a highly important component of food chains. Small chironomids are certainly heavily utilized by coho salmon juveniles, especially newly emerged and very young fry. These fish may often contain >30 small pupal chironomids (*Corynoneura*, *Thienemanniella*, and *Eukiefferiella*) per stomach (J. H. Mundie, pers. obs.). Moreover, emerging chironomids of all sizes are, because of their visibility on the water surface, significantly more available as food to coho salmon fry than are other drifting stages of aquatic insects (Mundie 1969, 1971).

The outcome of the experiment was a twofold increase in emerging insects in the absence of fish. The results were obtained under standardized conditions that departed from realism in several respects. Further mesocosm studies are required on several variables to improve prediction of effects of adding nutrients to streams.

Acknowledgements

The authors are indebted to I. Einer for his modelling skills and for substantial laboratory analysis, E. MacIsaac for chemical analyses of water, R. Bams and D. Crabtree for technical advice, T. Dickie for technical assistance, T. Burns and J. Wieczorek for field assistance, R. Kronlund for statistical advice, and Mrs. M. Sherry for word processing. Dr. N. H. Anderson kindly provided critical comments on the manuscript.

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Temperature and Lobster, *Homarus americanus*, Yield Relationships

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Campbell, A., D. J. Noakes, and R. W. Elner. 1991. Temperature and lobster, *Homarus americanus*, yield relationships. Can. J. Fish. Aquat. Sci. 48: 2073–2082.

Commercial landings of American lobster (*Homarus americanus*) have increased dramatically in many areas along the east coast of Canada since the late 1970's. Average annual sea surface temperatures (SST) were analysed to test if lobster landings were related to changes in the marine environment. Time series models were fitted to lobster landings and SST data from Maine (USA), Halifax County (Nova Scotia), and Charlotte County (New Brunswick). Including temperature in the models improved forecasting ability for lobster catches for Maine and Halifax but not Charlotte. In Maine, lobster landings in year *t* were related to SST in year *t*. In Halifax, however, landings were related to SST in the previous 4 yr but not by SST of the current year. Lower fishing effort levels for Halifax compared with Maine probably extended the yield from strong year-classes through a number of years. We surmise that an increase in sea temperature near Halifax during the early 1980's increased the survival and growth rates of lobster juveniles. This provided a strong pulse of recruits to the fishery throughout coastal Nova Scotia during the mid- to late 1980's. Similar increases in temperature and landings were not observed for Maine and Charlotte.

Les débarquements commerciaux du homard d'Amérique (*Homarus americanus*) ont augmenté radicalement en de nombreux endroits de la côte est du Canada depuis la fin des années 1970. On a analysé la température moyenne annuelle de la surface de la mer pour vérifier s'ils étaient liés à des changements du milieu marin. Des modèles de séries temporelles ont été appliqués aux débarquements de homards et à température de la surface de la mer au Maine (É.-U.), dans le comté de Halifax (Nouvelle-Écosse) et le comté de Charlotte (Nouveau-Brunswick). L'inclusion de la température dans les modèles a amélioré la capacité de prévoir les prises de homards du Maine et de Halifax, mais non de Charlotte. Dans le Maine, les débarquements de l'an *t* sont reliés à la température de l'eau de cette année. A Halifax cependant, les débarquements sont liés à la température des 4 années antérieures mais non à celle de l'année en cours. A Halifax, l'effort de pêche moindre qu'au Maine a probablement étalé le rendement des classes annuelles fortes sur plusieurs années. Nous posons l'hypothèse qu'une augmentation de la température de la mer près de Halifax au début des années 1980 a accru les taux de survie et de croissance des homards juvéniles, ce qui a donné des recrues fortes pour les pêches sur toutes les côtes néo-écossaises au milieu et à la fin de la décennie. On n'a pas observé d'augmentations semblables de la température et des débarquements au Maine et à Charlotte.

Received November 19, 1990
Accepted June 4, 1991
(JA807)

Reçu le 19 novembre 1990
Accepté le 4 juin 1991

Fluctuations in American lobster (*Homarus americanus*) landings over the past 100 yr have been attributed to a variety of environmental and biotic influences (see reviews by Campbell 1989; K. F. Drinkwater, G. C. Harding, W. P. Vass, and D. Gauthier, pers. comm; Elner and Campbell 1991; Pezzack 1991). During the late 1970's to late 1980's, lobster landings increased more or less in unison throughout most of the Gulf of St. Lawrence and coastal Nova Scotia (e.g. Fig. 1 and 2), suggesting that large unknown environmental forces may have acted during the same general time period (Drinkwater et al., pers. comm.; Elner and Campbell 1991; Pezzack 1991). Although record landings occurred in some local areas, other areas experienced lesser increases. Since mortality levels in most American lobster fisheries are considered high enough to remove 55–95% of recruited lobsters each year (Anthony 1980; Campbell 1980), the elevated yields are likely due, in part, to increases in lobster abundance.

One possible mechanism for increased lobster abundance involves the role of sea temperature in directly influencing the growth, survival, distribution, and catchability of lobster (Templeman 1936; McLeese and Wilder 1958; Campbell 1983; Harding et al. 1983). Various papers have shown positive relationships between temperature and *H. americanus* yields for the Gulf of Maine, Gulf of St. Lawrence, and Massachusetts (Dow 1969, 1977, 1978, 1980; Flowers and Saila 1972; Boudreault et al. 1977; Marchesseault 1977; Orach-Meza and Saila 1978; Fogarty 1988). Hitherto, no relationship between temperature and yield has been found for lobster fisheries along the Atlantic coast of Nova Scotia (Harding et al. 1983).

The two main objectives of this study were to (1) statistically test for a relationship between temperature and lobster landings and (2) explore the potential role of temperature in the increase in lobster landings on the Atlantic Coast of Nova Scotia. We applied time series analysis methods (cross-correlation analysis) to test for links between sea surface temperature (SST) and lobster landings for three areas (A) Halifax County, Nova Scotia, Canada, (B) Charlotte County, New Brunswick, Canada, and (C) the State of Maine, USA. Models were developed to determine if the inclusion of temperature provided more accurate one-year-ahead forecasts of yield. We show that there is a relationship between temperature and landings for Halifax and Maine, but not for Charlotte. From these results we hypothesize that a temperature increase during the late 1970's and

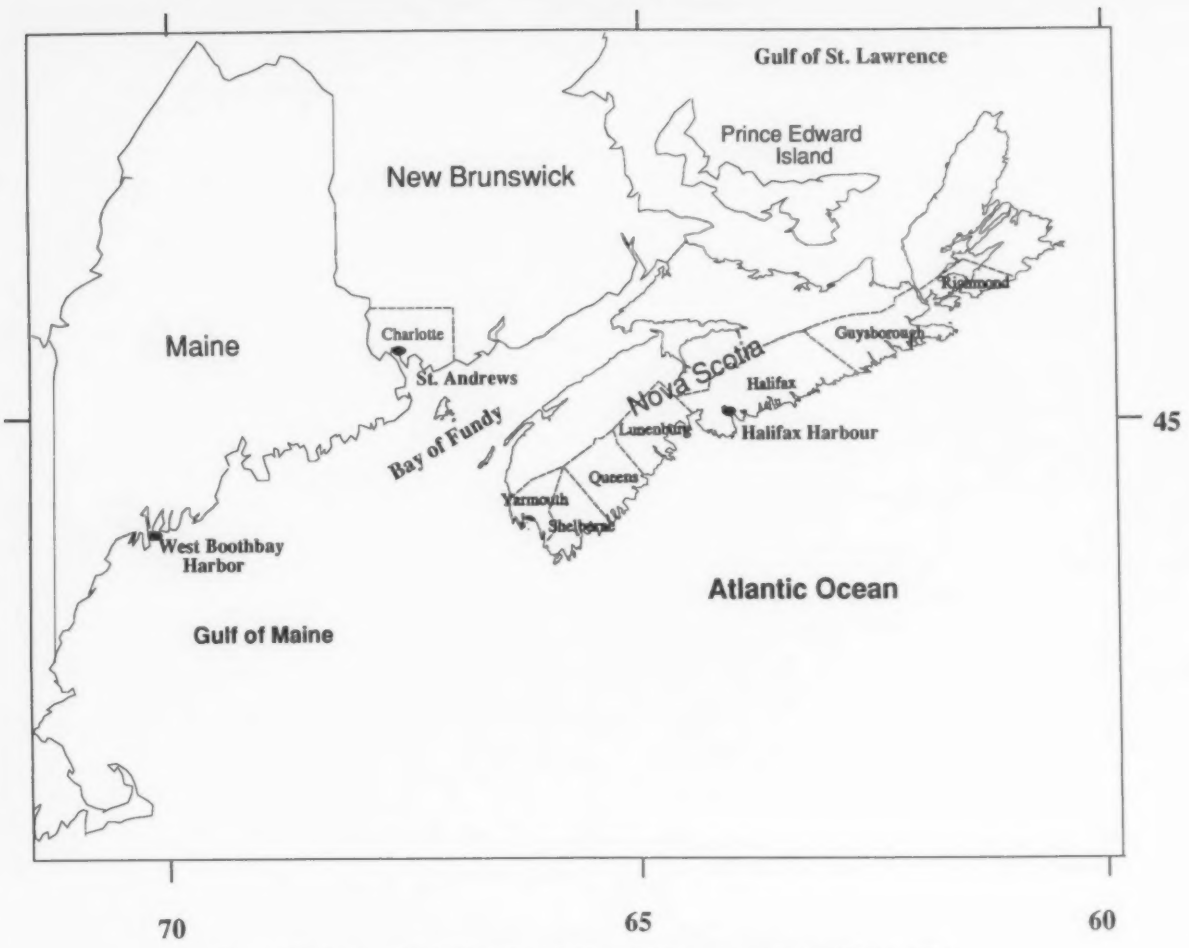

FIG. 1. Locality map for the Canadian Maritimes (with some counties) and Maine, USA.

early 1980's contributed to the increase in lobster landings observed along coastal Nova Scotia during the 1980's.

Material and Methods

Continuous series of annual total catch (metric tonnes) data for *H. americanus* fisheries were available from Maine during the period 1928–89 (J. S. Krouse, Maine Department of Marine Resources, West Boothbay Harbor, ME, pers. comm.), Halifax County, Nova Scotia, during 1893–1989, and Charlotte County (includes island of Grand Manan which make up about 20% of total lobster landings), New Brunswick, during 1921–89 (Fisheries Statistics, Halifax; Campbell and Mohn 1983). These landings data were used because continuous time series of SST data ($\geq$40 yr) were available only from positions within these areas. Although temperature can vary from location to location, seasonally, and with depth, for this paper we considered SST as a general indicator of annual trends for the local area. Mean annual SST (calculated from mean monthly data) were obtained from West Boothbay Harbor (Maine) for 1906–89 (Churchill 1988; J. S. Krouse, pers. comm.), St. Andrews (Charlotte County) for 1921–89 (Department of Fisheries and Oceans, St. Andrews Biological Station), and Halifax Harbour (Halifax County) for 1949–89 (Department of Fisheries and Oceans, Halifax). We interpolated data between months and years for the few months that temperature information was missing (i.e. 5 mo in 2 yr at West Boothbay, 9 mo in 8 yr at Halifax, and 7 mo in 2 yr at St. Andrews). Only mean annual SST were used in this paper. Seasonal average temperatures for the larval, juvenile growing, or fishing periods were not considered.

Two techniques, CuSum charts (Murdoch 1979) and cross-correlation analysis, were employed to examine relationships between lobster landings and SST. CuSum charts, a tool frequently utilized in statistical quality control, were used to visually assess if changes in lobster catches were associated with shifts in mean SST.

As exploratory data analysis techniques, these procedures often suggest useful avenues to pursue in terms of what type or functional form of plausible models to be entertained. There were no general trends in the raw SST data, so CuSum plots of SST were used. However, there were sufficient general trends in the annual lobster landings, so only the raw landings data were used for initial visual comparison. Linear (or log linear) relationships between lobster landings and SST were

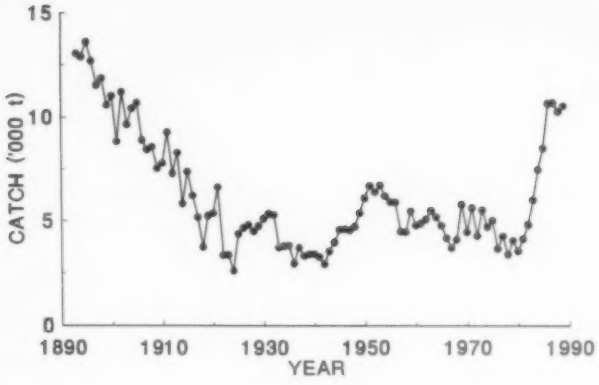

FIG. 2. Annual lobster landings for Coastal Nova Scotia from Yarmouth County to Richmond County.

investigated by examining cross-correlations between the residuals from models fitted to the catch and SST time series. The results of these analyses were then used to build transfer function models linking lobster catches and SST. CumSum and cross-correlation analyses are useful for determining if the two time series events are linked but may not provide detailed information regarding the nature of the link.

CuSum charts (Murdoch 1979) show the cumulative sum of differences between the observed phenomenon and a selected target value, such as the series mean. For a sequence of points, $X_1, X_2, \ldots$, the CuSum is given as

$$(1a)\quad S_1 = (X_1 - \alpha)$$

$$(1b)\quad S_2 = (X_1 - \alpha) + (X_2 - \alpha) = S_1 + (X_2 - \alpha)$$

$$\vdots$$

$$(1c)\quad S_r = \sum_{i=1}^{r} (X_i - \alpha) = S_{r-1} + (X_r - \alpha)$$

where α is a constant reference or target value. If the series fluctuates randomly about the target value, then the slope of the CuSum plot does not change with time. However, the slope of the CuSum plot will increase or decrease in response to changes in the mean level of the series.

Since the CuSum is essentially an integration, the shape of the plot provides an indication of the type of change in the mean level of the series. For example, step changes in the mean level of a process will result in slope changes in the CuSum, linear changes in the mean result in a quadratic CuSum, and so forth. These plots provide useful information on the types of changes in the mean level of each process. If changes in the slopes occur near the same time for each time series being considered, this may indicate that the series are causally related.

Various procedures are available to determine the type of relationship between two time series (e.g. Granger 1969a, 1969b; Haugh 1976; Haugh and Box 1977; Pierce and Haugh 1977; Hipel et al. 1982; Hipel et al. 1985). In this study, univariate time series models (Box and Jenkins 1976) were fitted to each landing and temperature series (prewhitening). Cross-correlations were then calculated using the model residuals to identify possible linear (or loglinear) relationships between lobster landings and SST. Employing the model residuals instead of the raw or transformed data minimizes the changes of obtaining spurious cross-correlations. A similar procedure was used by Fogarty (1988) in his analysis of Maine lobster landings and temperature data.

Once tentative links between lobster landings and SST were identified with cross-correlation analysis, transfer function models relating these two variables were constructed. Transfer function models (Box and Jenkins 1976) were of the form

$$(2)\quad (y_t - \mu_y) = \sum_{i=1}^{m} \omega_i(B)\{\delta_i(B)\}^{-1} \cdot B^{b_i} \cdot (x_{t,i} - \mu_{x_i}) + \theta(B)\{\phi(B)\}^{-1}\sigma\epsilon_t$$

where t is discrete time, m is the number of transfer functions in the model, y_t is the response or output variable, μ_y is the mean of the y_t series, ϵ_t is the error term or model residual (usually assumed to be normal and independently distributed with mean zero and variance 1), and σ is the standard deviation of the model residuals. If necessary, both the response variable, y_t, and the input covariate series, $x_{t,i}$, can be transformed (Box and Cox 1964) so that model residuals reasonably satisfy the normality assumption.

The transfer function components of the model,

$$(3)\quad \omega_i(B)\{\delta_i(B)\}^{-1}, \qquad i = 1,\ldots,m,$$

are represented in terms of the finite-order polynomials

$$(4)\quad \omega_i(B) = \omega_{0i} - \omega_{1i}B - \omega_{2i}B^2 - \ldots - \omega_{u_i i}B^{u_i}$$

and

$$(5)\quad \delta_i(B) = 1 - \delta_{1i}B - \delta_{2i}B^2 - \ldots - \delta_{r_i i}B^{r_i}$$

where $\omega_{0i}, \omega_{1i}, \ldots, \omega_{u_i i}$ and $\delta_{1i}, \delta_{2i}, \ldots, \delta_{r_i i}$ $(i = 1, 2, \ldots, m)$ are the parameters to be estimated and u_i and r_i are the orders of the polynomials $\omega_i(B)$ and $\delta_i(B)$, respectively. The terms B^{b_i} is used to account for any delay in response associated with the ith covariate series. The covariate times series, $x_{t,i}$, are variables related to the output variables, y_t, in some fashion. Although only SST's were considered in this study, a number of biotic and abiotic variables would be potential candidates if plausible links could be identified (Stocker and Hilborn 1981; Shepherd et al. 1984; Stocker and Noakes 1988).

The stochastic noise component of the model (2), $\theta(B)\{\phi(B)\}^{-1}\sigma\epsilon_t$, is used to model the autocorrelation structure associated with the data. This term includes a moving average component of order q (MA(q)), $\theta(B)$, and an autoregressive operator of order p (AR(p)), $\phi(B)$, given by

$$(6)\quad \theta(B) = 1 - \theta_1 B - \theta_2 B^2 - \ldots - \theta_q B^q$$

and

$$(7)\quad \phi(B) = 1 - \phi_1 B - \phi_2 B^2 - \ldots - \phi_p B^p$$

respectively. The combined term is referred to as an ARMA(p,q) process. In an AR process, the observation at time t is related linearly to the observations before time t. A MA process expresses the response variable in terms of the previous errors. Mixtures of AR and MA processes result in a convenient and compact method of describing the autocorrelation structure present in a particular time series.

As a final test, we used the univariate and transfer function models developed in the previous step to generate one-step

(year)-ahead forecasts of lobster catch at each location. Each series was shortened by eliminating the last 10 observations so that independent data were used to test forecasting performance. Univariate and transfer function models were then fitted to each series and used to forecast catch for the next year. Model parameters were updated each year and the process was repeated 10 times until 10 one-year-ahead forecasts of catch had been generated for each model and location. The transfer function model for Maine included the current year's SST (i.e. lag 0). Since this quantity was not yet observed, a univariate time series model was used to forecast the current year's temperature. The forecasted temperature was then included in the model used to forecast landings. For comparison, the form of the model proposed by Fogarty (1988) was included in the analysis. The performance of the various models was compared using root mean square error (RMSE), mean absolute deviation (MAD), and mean absolute percent error (MAPE) criteria.

Caution is required when interpreting the time series analyses, since changes in effort may have influenced the annual lobster landings trends. There are no consistent measurements to provide a long time series of effective fishing effort or mortality. Consequently, we could not separate the effects of effort and temperature on landings in the time series analyses. The number of traps is a poor indication of effective effort to compare over time and between locations because the efficiency of traps can vary seasonally depending on different activity levels (McLeese and Wilder 1958) and physiological conditions of lobsters and market demands influencing intensity of daily trap hauls (see reviews by Elner 1980; Harding et al. 1983). We assumed that trap effort intensity was sufficiently high to cause 55–95% mortality allowing landings to provide some indication of lobster abundance (Thomas 1973; Anthony 1980; Campbell 1980, 1990; Miller et al. 1987). Increased lobster abundance may also influence the increase of effort (Pringle and Duggan 1984; Campbell 1990).

Results

Annual landings for both Halifax and Charlotte peaked around the turn of the century at about 2200 and 1200 t, respectively, decreased steadily until the 1920's, and have fluctuated thereafter (Fig. 3A, 4A). In Maine, landings were low until the early 1940's at which time they tripled to about 9000 t (Fig. 5A). There is evidence of sporadic increases in landings in all three areas, but they are most notable in the Halifax time series (Fig. 3A). Increases probably correspond to the recruitment of a strong year-class, or classes, into the fishery and subsequent harvesting over the following 2–3 yr.

Mean annual SST's in all three areas fluctuated considerably showing little evidence of significant general trends over time (Fig. 3–5). Average annual temperatures over the periods of interest were 8.3, 7.8, and 7.0°C at West Boothbay, Halifax, and St. Andrews, respectively. Differences between minimum and maximum average annual temperatures were largest at West Boothbay (4.8°C), while temperature variations were similar at both Halifax and St. Andrews (3.0 and 3.1°C, respectively).

CuSum plots for SST's suggest that shifts in lobster landings may be partially related to variations in mean temperature (Fig. 3C, 4C, 5C). General trends of the temperature CuSum plots approximately coincide with increases or decreases in lobster landings (Fig. 3A, 4A, 5A). Adjusting for scale, the pattern of lobster landings for both Maine and Charlotte and the shapes of the SST CuSum plots parallel each other closely

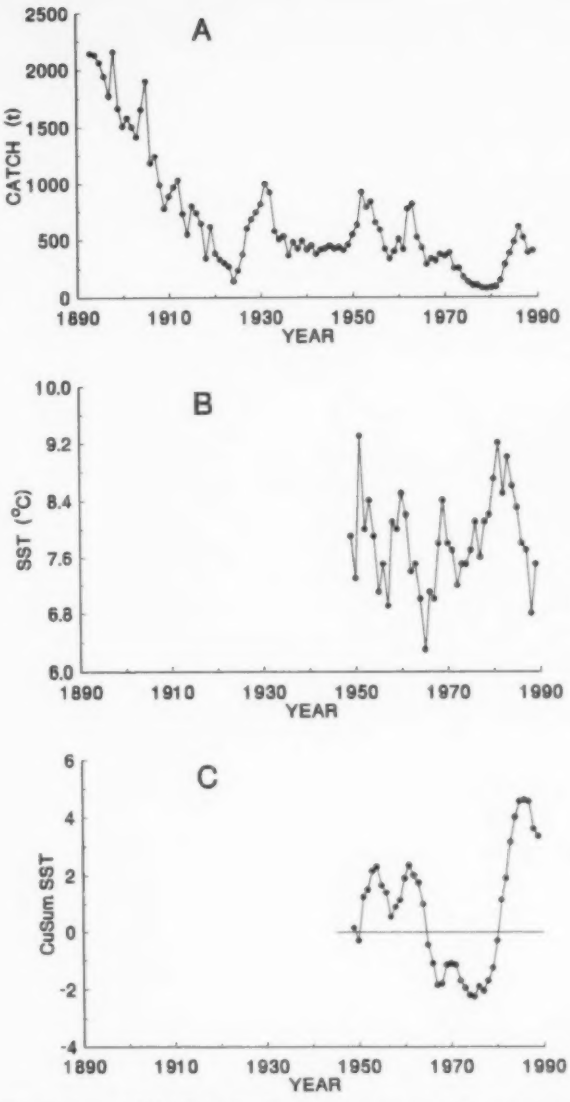

FIG. 3. Annual (A) lobster landings, (B) mean sea surface temperatures (SST), and (C) CuSum of SST for Halifax County (temperatures from Halifax Harbour), Nova Scotia, Canada.

(Fig. 4, 5). Landings increased nearly threefold in both locations in the 1940's and have remained elevated since that time. Both events appear to be linked to increases in mean SST's in the fishing areas. For SST in Charlotte and Maine, the distinct increase in the slope of the CuSum plot (Fig. 4C, 5C) in the late 1940's to early 1950's indicates the start of a warming trend. The timing of the event generally coincides with a sharp increase in lobster landings in both Charlotte and Maine (Fig. 4A, 5A) which may have been partly due to expansion of the fishery. However, the two events, a warming trend and postwar expansion of the fishery, are compounded making separation of their effects impossible.

Links between changes in mean SST and lobster landings from Halifax are less apparent than for the other two areas. The

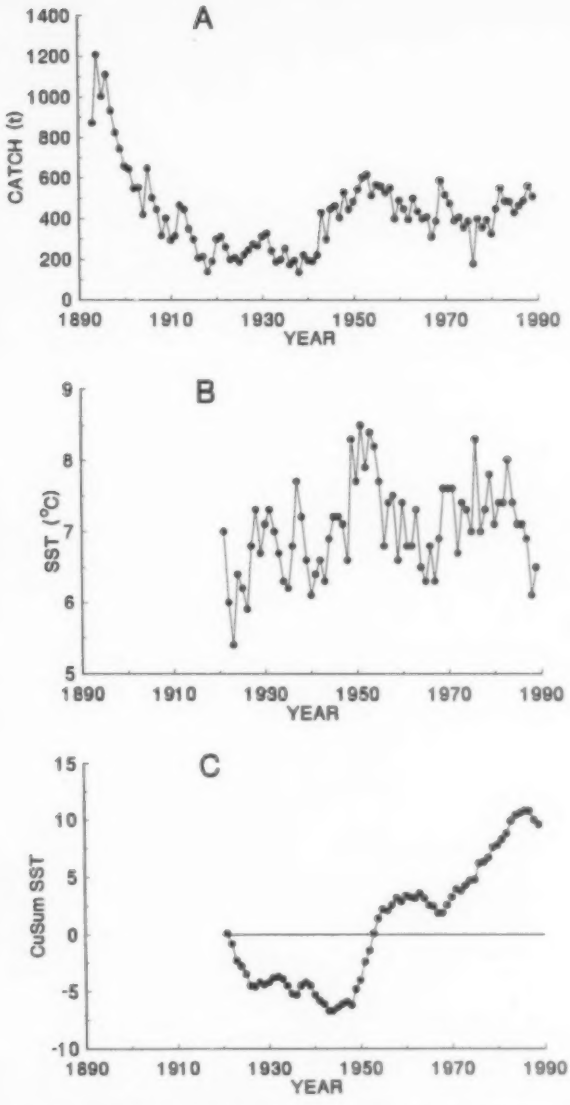

FIG. 4. Annual (A) lobster landings, (B) mean sea surface temperatures (SST), and (C) CuSum of SST for Charlotte County (temperatures from St. Andrews), New Brunswick, Canada.

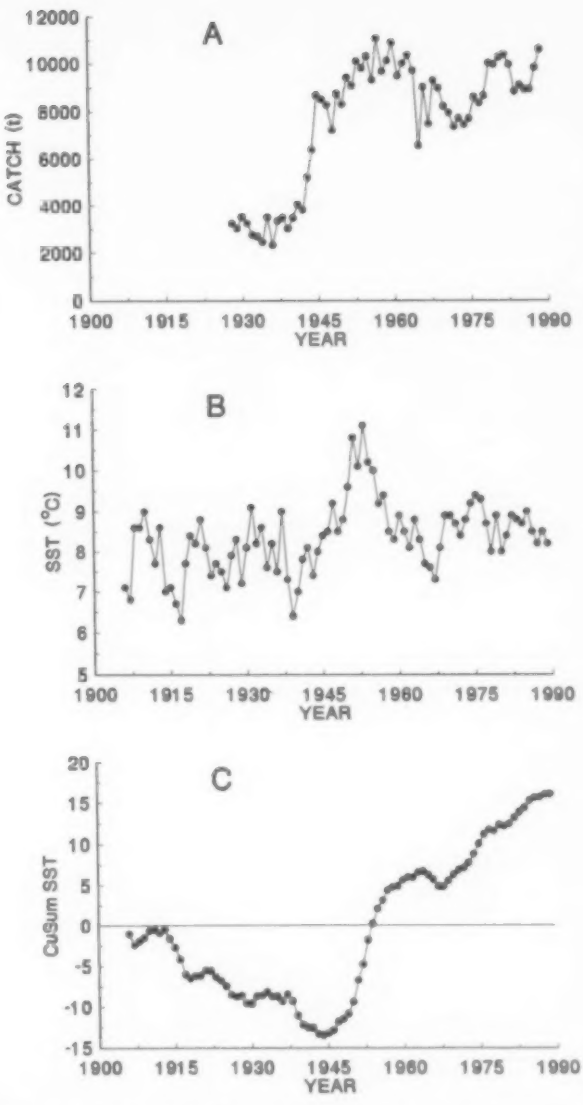

FIG. 5. Annual (A) lobster landings, (B) mean sea surface temperatures (SST), and (C) CuSum of SST for Maine (temperatures from Boothbay Harbour), USA.

jagged appearance of the Halifax SST CuSum (Fig. 3B) suggests frequent shifts in SST between short periods (5–10 yr duration) of warmer and cooler temperatures. Elevations in mean SST, as indicated by increases in the slope of the SST CuSum plot (Fig. 3C), occurred in the early and late 1950's and late 1970's to early 1980's. After each increase in mean SST, there was a corresponding rise in lobster landings that decayed because stocks were depleted and/or either effort or temperatures fell (Fig. 3A).

The time series models fitted to the lobster landings and SST data are presented in Table 1. All available data were used to estimate the parameters of the univariate models. When the landing and SST series were of different length, the longer series was truncated prior to fitting the transfer function models. In all cases, lobster landings were differenced once to account for the obvious shifts in mean level over time. If necessary, the data were transformed to ensure that the model residuals reasonably satisfied the normality assumption. Lobster landings from Halifax and Maine were $\log_e$ transformed (Box–Cox $\lambda = 0$), while an approximate square root transformation (Box–Cox $\lambda = 0.5$) was employed for lobster landings from Charlotte. No transformation (Box–Cox $\lambda = 1$) was employed for the univariate models fitted to the SST data. In all cases, the residuals were checked to ensure that they were uncorrelated and approximated a normal distribution.

Model residuals from each of the univariate models fitted to the lobster landing and SST time series were used to estimate cross-correlations functions (Fig. 6). Not unexpectedly, the

TABLE 1. Time series models fitted to the lobster catch and SST data. Values in parentheses are the standard errors of the estimated parameters and λ is the exponent of the Box–Cox transformation. SE = ±1 standard error; SST = sea surface temperature; TFN = transfer function noise; AIC = Akaike (1974) information criterion.

Location	Data type	Period	Parameters (SE)
Halifax	Landings $\lambda = 0$	1893–1989	$\phi_5 = -0.291(0.098)$ $\sigma^2 = 0.063$ AIC = 949.2
	Landings $\lambda = 0$	1949–89	$\phi_5 = -0.356(0.148)$ $\sigma^2 = 0.063$ AIC = 369.7
	SST $\lambda = 1$	1949–89	$\phi_1 = 0.297(0.120)$ $\phi_2 = 0.681(0.150)$ $\phi_4 = -0.694(0.153)$ $\phi_5 = 0.265(0.141)$ $\sigma^2 = 0.222$ AIC = −49.4
	TFN (SST at lags 3 and 4) $\lambda = 0$ (landings) $\lambda = 1$ (SST)	1949–89	$\omega_{31} = 0.143(0.069)$ $\omega_{41} = 0.127(0.069)$ $\sigma^2 = 0.065$ AIC = 368.0
Charlotte	Landings $\lambda = 0.5$	1921–89	$\phi_3 = -0.404(0.111)$ $\sigma^2 = 15.4$ AIC = 600.6
	SST (St. Andrews) $\lambda = 1$	1921–89	$\phi_3 = 0.546(0.101)$ $\sigma^2 = 0.281$ AIC = −83.4
	TFN $\lambda = 0.5$ (landings) $\lambda = 1$ (SST)	1921–89	$\phi_1 = -0.424(0.110)$ $\omega_{51} = -1.55\ (0.852)$ $\sigma^2 = 15.1$ AIC = 596.0
Maine	Landings $\lambda = 0$	1928–88	$\phi_3 = -0.407(0.118)$ $\sigma^2 = 0.019$ AIC = 842.5
	SST $\lambda = 1$	1906–88	$\phi_3 = 0.691(0.079)$ $\sigma^2 = 0.422$ AIC = −66.9
	Fogarty (1988) TFN (ln (SST) at lags 0 and 1)	1928–88	$\phi_1 = -0.292(0.124)$ $\omega_{01} = 0.781(0.207)$ $\omega_{11} = -0.368(0.203)$ $\sigma^2 = 0.015$ AIC = 831.2
	TFN (SST at lag 0 only)	1928–88	$\phi_1 = -0.301(0.123)$ $\omega_{01} = 0.091(0.025)$ $\sigma^2 = 0.016$ AIC = 833.2
	TFN (SST at lags 0 and 1)	1928–88	$\phi_1 = -0.302(0.123)$ $\omega_{01} = 0.090(0.025)$ $\omega_{11} = -0.043(0.025)$ $\sigma^2 = 0.015$ AIC = 832.0

cross-correlation function for Maine catches and SST's was similar to the one estimated by Fogarty (1988). Differences between the two are, in part, due to slight variations in the data and estimation procedures used in the two studies.

Significant positive correlations were found between SST and lobster landings for Halifax and Maine. For the Halifax data, landings in year t were positively related to SST in the previous 4 yr (i.e. $t - 1$, $t - 3$, and $t - 4$) but were not statistically significant in either the current year or $t - 2$ (Fig. 6A). No strong relationship was found between lobster landings for Charlotte and SST at St. Andrews; at best, there was a negative correlation at $t - 5$ (Fig. 6B). However, when SST at lag 5 was included in the model for lobster landings in Charlotte, the parameter associated with this term was not significantly different from zero. In Maine, lobster catches were positively correlated with SST in the same year (Fig. 6C). Although not sta-

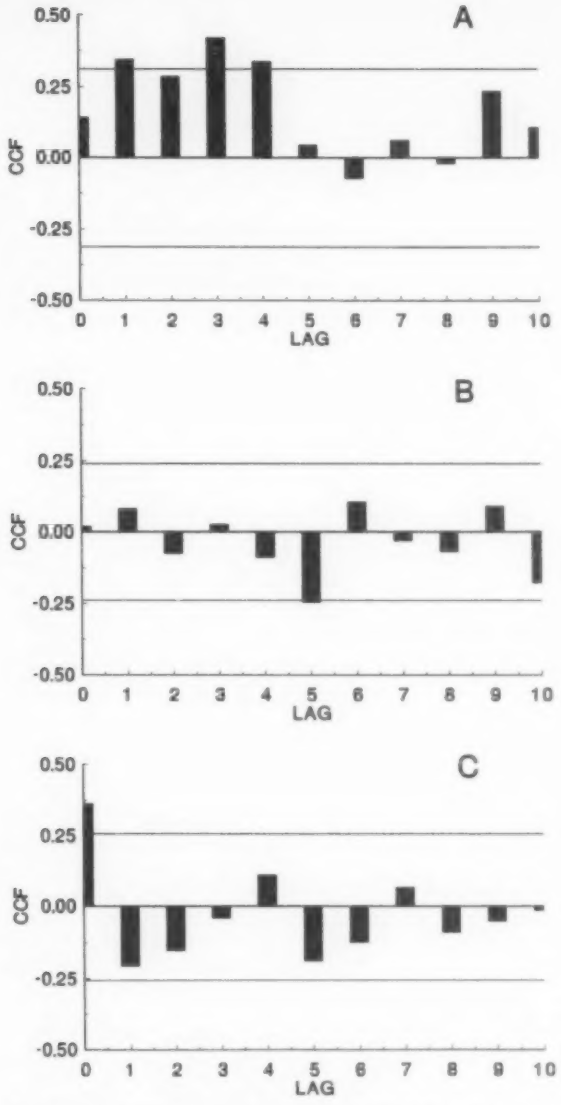

FIG. 6. Cross-correlation function for lobster landings and sea surface temperature data from (A) Halifax, N.S., (B) Charlotte, N.B., and (C) Maine, USA. The horizontal lines represent approximate 95% confidence intervals.

tistically significant, Maine lobster landings in year t may also, as suggested by Fogarty (1988), be negatively correlated with the previous year's SST ($t - 1$).

The estimated cross-correlations were used to identify transfer function models linking SST and lobster landings (Table 1). For comparative purposes, the transfer function model identified by Fogarty (1988) was fitted to the Maine data. Using the residual variance as a comparative measure of fit, the transfer function noise (TFN) models were slightly better than univariate models fitted to landings in Halifax and Maine but not Charlotte. The Akaike information criterion (AIC; Akaike 1974) provides a combined measure of statistical fit and model parsimony and was also employed to assess model adequacy. Lower AIC values indicate improved model fits. In general, incorporating SST into the model lowered the AIC's. The most significant improvement in model fit was for Maine where the inclusion of SST reduced the AIC by approximately 10 units (Table 1). Lower AIC's do not always imply improved ability to forecast future events (Noakes et al. 1985), but performance is likely to improve as differences in AIC increase.

TABLE 2. Root mean square error (RMSE), mean absolute deviation, and mean absolute percent error of the 10 one-step-ahead forecasts generated by the various univariate and transfer function noise (TFN) models. Lower values of each criterion indicate improved forecast performance.

Location	Model	RMSE (t)	MAD (t)	MAPE
Halifax	Univariate	74	60	17.8
	TFN	66	57	17.3
Charlotte	Univariate	70	56	11.7
	TFN	79	64	13.6
Maine	Univariate	710	534	5.6
	Fogarty(1988)			
	TFN (ln(SST) at lags 0 and 1)	577	410	5.4
	TFN (SST at lag 0 only)	667	462	4.8
	TFN (SST at lags 0 and 1)	562	396	4.2

One-step-ahead forecasts generated using the TFN models fitted to Maine lobster landings were generally better than those produced by the univariate model. On average, RMSE's, MAD's, and MAPE's for the 10 TFN forecasts were 15–20% less than for the univariate model (Table 2). The TFN forecasts for the Halifax data were only slightly better than forecasts generated by the univariate models, with reduction in the three criteria being on the order of 5–10%. For Charlotte landings, the univariate model forecasted about 10% better than the TFN model. Thus, including SST in the model did not markedly improve our ability to forecast Charlotte lobster landings.

Discussion

Time series analyses were successfully used to explore the relationship between temperature and catch in three lobster fisheries. The cross-correlation analyses indicated that lobster landings for (1) Halifax were significantly correlated with SST lagged 1–4 yr (except 2 yr), (2) Charlotte were virtually independent of SST with a minor term at lag 5 yr, and (3) Maine were correlated with SST at lag 0 (Fig. 4). Correlations after filtering are usually considerably lower than those for the original series of temperature and yield (Fogarty 1988), since prewhitening is a conservative procedure (Box and Pierce 1970). The univariate and transfer function models indicated that including SST improved our ability to forecast landings 1 yr ahead for Halifax and Maine, but not for Charlotte.

A major assumption of this paper is that landings directly reflect available commercial biomass through relatively high fishing effort. As fishing mortality rises, landings become an increasingly useful proxy for population size and recruitment. Thus, temperature effects can be linked more directly to changes in stock size under high exploitation regimes. Unfortunately, long-term time series of mortality (fishing and natural) rates are not available for lobster in the areas studied. Mortality estimates that are available vary between years and areas from 55 to 95% (Paloheimo 1963; Thomas 1973; Anthony 1980; Camp-

TABLE 3. Cross-correlation functions for lobster landings from different counties of coastal Nova Scotia and sea surface temperature data from Halifax.

Lag	Lunenburg	Queens	Richmond to Yarmouth
0	0.26	0.15	0.28
1	0.32	0.16	0.21
2	0.22	0.25	0.16
3	0.26	0.26	0.14
4	0.13	0.24	0.04
5	0.09	0.33	0.13
6	−0.33	0.05	−0.12

bell 1980, 1990; Miller et al. 1987). Mean mortality rates for lobsters from areas near Charlotte and Halifax were similar (approximately 73%) during 1983–85 (Miller et al. 1987; Campbell 1991). However, during the mid- to late 1970's, mortality rates in southwestern Nova Scotia may have been as low as 55% (Campbell 1990). Mortality rates for lobsters recruited in the Maine fishery varied from 72 to 95% during 1967–75 (Thomas 1973; Anthony 1980). There is no fishing season or trap limit regulation for the Maine lobster fishery (Kelly 1990) whereas there are for the Nova Scotia and Bay of Fundy lobster fisheries (Campbell 1989).

CuSum plots of SST were useful in detecting changes in process level. Like cross-correlations, CuSum's should be viewed as exploratory data analysis or identification tools that merely suggest useful avenues to pursue and types of models to consider. The positive slopes of CuSum plots indicate periods of warmer SST's, and the magnitude of the slope indicates the degree of warming. A steeper slope indicates a more rapid warming. SST's increased more rapidly near Halifax than for Charlotte or Maine during the late 1970's to 1980's (Fig. 3C, 4C, 5C). The slopes of the SST CuSum's for Charlotte and Maine were greater during the 1940's and 1950's than the 1980's (Fig. 4C, 5C). Landings in both Maine and Charlotte increased slightly in the 1980's but not as dramatically as in the 1940's and 1950's (Fig. 4A, 5A). There was a cooling period in the 1960's and early 1970's (i.e. CuSum slopes were less) and landings decreased slightly for both Maine and Charlotte.

Our principal finding is the significant correlation between lobster landings and SST from Halifax. The results suggest that temperature changes may have influenced the increases in lobster landings observed off Halifax during the 1950's, 1960's, and 1980's (Fig. 3). In contrast, Harding et al. (1983) did not detect statistically significant correlations (at 5% level) between Halifax SST and landings from Halifax to Lunenburg County and Queens County, Nova Scotia. We conducted a comparable initial time series of cross-correlation of Halifax SST with landings from Lunenburg and Queens and coastal Nova Scotia (combining Richmond to Yarmouth counties) during 1949–89 after prewhitening the data. The pattern for Lunenburg was similar to that observed for Halifax landings, the exception being the cross-correlation for Queens at lag 5 yr (Table 3). Also, smaller values for Lunenberg and Queens and coastal Nova Scotia compared with those for Halifax for lag 1–4 yr could be a result of increased distance from the SST measurement site (i.e. local temperatures may differ slightly compared with those for Halifax).

A warming trend during the early 1980's appears, at least partly, to be responsible for increased lobster landings throughout Nova Scotia. Although we do not have temperature records comparable with those for Halifax for the rest of the coast, lobster landings show a similar greater than threefold increase in magnitude for most Nova Scotian stocks during the 1980's. Coincidentally, a pathogen devastated green sea urchin (*Strongylocentrotus droebachiensis*), populations along the entire Atlantic shore of Nova Scotia during 1980–83 (Jones and Scheibling 1985; Elner and Vadas 1990). Both Scheibling and Stephenson (1984) and Miller (1985) documented concomitant increases in SST, utilizing Halifax Harbour records and remote sensing data from satellites, respectively, and hypothesized that elevated temperatures had been critical to transmission of the disease (but see also Margosian et al. 1987). In comparison, the warming trend for Halifax (Fig. 3C) was markedly greater than that for Maine and Charlotte during the 1980's (Fig. 4C, 5C).

An immediate temperature effect (lag 0–1 yr) on annual Maine lobster landings was indicated by this study and Fogarty (1988). Temperature controls lobster activity and consequently catchability (McLeese and Wilder 1958; Paloheimo 1963) causing short-term (lag 0–1 yr) changes in yield, assuming relatively constant and high levels of exploitation. Similarly, increases in the annual probability of molting for prerecruit lobsters have been linked to rises in sea temperature (Ennis et al. 1982; Campbell 1983) acting, also, to augment recruitment and yield over the short term. In addition, the single significant 0-yr lag for Maine suggests that fishing mortality was high enough that each recruitment pulse was harvested in the same year (Fogarty 1988) rather than being spread over an extended time period (i.e. 3–4 yr as for Halifax).

Trends in annual landings from Charlotte were virtually independent of annual mean SST, except for a spurious negative value at lag 5 yr for which we have no explanation. The lack of correlation suggests that recruitment is attributable to immigration from other areas. Sources of lobster larvae settling on any of the fishing grounds are unknown (Campbell 1985; Elner and Campbell 1991), and mature lobsters, making up 4–20% of total catch (depending on location) (Campbell and Duggan 1980; Campbell 1986), are known to move in and out of the Charlotte area (Campbell and Stasko 1986). However, immature recruit sized lobsters throughout Maine and Atlantic Canada usually move only small distances (Krouse 1981; Campbell and Stasko 1986; Miller et al. 1989). Variations in fishing effort might also obscure the influence of the environment on yields (Drinkwater and Myers 1987).

Temperature probably acts with other factors to cause observed longer term (>1 yr) lags between landing and temperature relationships (Fogarty 1988; Drinkwater et al., pers. comm.; Pezzack 1991). These underlying mechanisms may include temperature which produces increased yields at lags of 2–6 yr as a result of enhanced growth and survival rates of early life history stages of *H. americanus* (Huntsman 1923; Templeman 1936; Harding et al. 1983; Aiken and Waddy 1986; Ennis 1986; Fogarty 1988). Temperature has also been shown to influence growth of juveniles of other crustacean species (e.g. the red king crab (*Paralithodes camtschatica*)) and subsequent recruitment (Stevens 1990). Differential juvenile growth rates could spread a cohort recruiting into the lobster fishery over several years (Caddy 1977). The results of the cross-correlation analyses for Halifax are consistent with the assumption that 3–4 yr classes were being exploited and that fishing effort levels were such that years of large recruitment could not be fully exploited in a single year (i.e. a large year-class recruitment

pulse would influence landings over 3 or 4 yr if total annual mortality was not excessive for recruited lobsters). Also, reduced fishing mortality in some areas during the late 1970's and early 1980's (Pringle and Duggan 1984; Miller et al. 1987) could have allowed more adult females to survive, favouring increased egg production and swollen recruitment into the fishery 5–8 yr later (Campbell 1990).

While many factors may contribute to the magnitude of lobster recruitment, physical environmental controls (such as temperature) may largely overide the range of possible density-dependent effects on the early life history stages of *H. americanus* (Elner and Campbell 1991; Wahle and Steneck 1991). Our findings show that short-term predictions on lobster yield can be made using SST data for certain local areas such as Maine and Halifax. However, hypotheses need to be formulated and tested to elucidate the underlying ecological mechanisms for the correlations and predictions. The fact that SST data are not predictive for areas such as Charlotte indicates that interarea comparisons of factors such as oceanography and climate could yield important clues to the recruitment process (see also Frank et al. 1990).

Acknowledgements

We thank W. Carolsfeld, M. D. Eagles, and R. I. Perry for assistance and advice, J. Krouse and K. M. Kelly for providing SST and lobster landings data for Maine, D. Pierce for typing, and J. T. Addison, M. J. Fogarty, R. J. Miller, R. I. Perry, and B. D. Smith for comments on this paper.

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Effects of Salinity on Growth, Metabolism, and Ion Regulation in Juvenile Rainbow and Steelhead Trout (*Oncorhynchus mykiss*) and Fall Chinook Salmon (*Oncorhynchus tshawytscha*)

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Morgan, J. D., and G. K. Iwama. 1991. Effects of salinity on growth, metabolism, and ion regulation in juvenile rainbow and steelhead trout (*Oncorhynchus mykiss*) and fall chinook salmon (*Oncorhynchus tshawytscha*). Can. J. Fish. Aquat. Sci. 48: 2083–2094.

Rainbow and steelhead trout (*Oncorhynchus mykiss*) and fall chinook salmon (*Oncorhynchus tshawytscha*) fry were acclimated to a range of salinities to test the hypothesis that the energy requirements for ion regulation would be minimal at an isotonic water salinity (8–10 ppt). Survival, growth, metabolic rate, plasma Na^+ and Cl^- concentrations, and seawater adaptability were measured for 5–12 wk, depending on the species. Growth of all three species was highest in fresh water and declined with increasing salinity. Metabolic rates increased with salinity and were inversely correlated with growth rates. Isotonic salinity, therefore, did not offer significant metabolic or growth advantages to rainbow, steelhead, and chinook fry. While plasma Na^+ and Cl^- concentrations varied among groups, chinook fry tended to better maintain ionic homeostasis at higher salinities than the trout. Acclimation to the various dilute salinities did not influence the seawater adaptability of juvenile steelhead trout or chinook salmon. Our results indicate that optimal salinities for growth and metabolic rates were influenced by species and life history stage. The metabolic rate data suggested that the energetic cost of ionic regulation increased with salinity, but attempts to quantify this cost were probably affected by other metabolic processes which responded to changes in salinity.

On a acclimaté des alevins de truite arc-en-ciel et de truite arc-en-ciel anadrome (*Oncorhynchus mykiss*) et de saumon quinnat (*Oncorhynchus tshawytscha*) à diverses salinités afin de vérifier l'hypothèse selon laquelle les besoins énergétiques de la régulation ionique seraient faibles à une salinité isotonique (8–10 ppm). Puis on a quantifié le taux de survie, le taux de croissance, le rythme métabolique, les teneurs en Na^+ et en Cl^- du plasma ainsi que le niveau d'adaptation au milieu marin pendant 5 à 12 sem, selon l'espèce. Le taux de croissance des trois espèces était plus élevé en eau douce et a diminué en fonction d'une augmentation de la salinité. Par contre, leur rythme métabolique a augmenté en fonction de la salinité et était en corrélation inverse avec leur taux de croissance. La salinité isotonique n'a toutefois pas offert d'avantages significatifs au niveau métabolique. Quoique les teneurs en Na^+ et en Cl^- dans le plasma variaient d'un groupe à l'autre, les alevins de saumon quinnat avaient tendance à maintenir une meilleure homéostasie ionique à des salinités élevées que les alevins de truite. L'acclimatation aux diverses salinités diluées n'a pas influé sur la capacité d'adaptation des juvéniles de truite arc-en-ciel anadrome et de saumon quinnat au milieu marin. Les résultats de l'étude révèlent que l'espèce et le stade du cycle vital influent sur les salinités optimales en ce qui concerne la croissance et le métabolisme. Les données pertinentes au rythme métabolique portent à croire que le coût énergétique de la régulation ionique augmente en fonction de la salinité, mais les essais de quantification de ce coût ont probablement été affectés par d'autres processus métaboliques qui ont réagi aux variations de la salinité.

Received November 30, 1990
Accepted May 2, 1991
(JA830)

Reçu le 30 novembre 1990
Accepté le 2 mai 1991

There are conflicting reports in the literature regarding the effects of isotonic salinity (i.e. 8–10 ppt; Brett 1979) on the growth and metabolic rate of salmonids. Both Canagaratnam (1959) and Otto (1971) found that growth rates of presmolt coho salmon (*Oncorhynchus kisutch*) were higher between 5 and 12 ppt than in freshwater. Measurements of oxygen consumption in rainbow trout (*Oncorhynchus mykiss*) further showed a reduction in metabolic rate (20–28%) in isotonic salinity relative to fresh water and seawater (Rao 1968). These studies support the hypothesis that the energetic cost of ion regulation is lowest in an isotonic environment, where the ionic gradients between blood and water are minimal, and that this energy savings is substantial enough to increase growth. Other studies, however, fail to show isotonic salinity as the point of maximal growth in salmonids; growth rates were highest in fresh water in studies by Shaw et al. (1975), Clarke et al. (1981), McKay and Gjerde (1985), and McCormick et al. (1989c). A theoretical estimate of the energetic cost of osmoregulation in salmonids suggests that it would be very low, being less than 1% of resting metabolism (Eddy 1982). Accordingly, changes in metabolic rate of that magnitude would be very difficult to measure accurately. Studies such as that of Bullivant (1961), who reported no significant differences between the metabolic rates of yearling chinook salmon (*Oncorhynchus tshawytscha*) reared in fresh water, half-strength seawater, and full-strength seawater, support those theoretical estimates.

Few studies have made simultaneous measurements on growth and metabolic rates of salmonids in relation to salinity.

To study those relationships, we examined growth, metabolic rates, and ionic regulation in juvenile rainbow and steelhead trout and fall chinook salmon acclimated to a range of salinities, with one near isotonic. These species were chosen to investigate the possible effects of different life history patterns on growth and metabolic responses to environmental salinity. The steelhead trout is an anadromous form of the rainbow trout, usually spending 2 yr in fresh water before migrating to the sea (Scott and Crossman 1973). Fall chinook salmon are also anadromous, but spend a shorter period of time in fresh water than steelhead trout, migrating seaward within 3 mo of emergence (Lister and Walker 1966).

Materials and Methods

Fish Stocks

Domesticated rainbow trout fry were obtained from Sun Valley Trout Farms, Mission, B.C., in November 1988. Steelhead trout and fall chinook salmon eggs were taken at the Big Qualicum River hatchery on Vancouver Island in April and November 1989, respectively, and incubated to the swim-up stage at the Pacific Biological Station in Nanaimo. All three stocks were transferred to the E.V.S. Consultants Marine Laboratory in North Vancouver and were held in fibreglass tanks with running fresh water for up to 3 wk prior to experimentation.

Growth Experiments

The experimental design for each growth trial is shown in Table 1. At the start of the experiments, the fry were lightly anesthetized in 2-phenoxyethanol (0.5 $mL \cdot L^{-1}$), measured for length (to the nearest millimetre) and weight (to the nearest 0.01 g), and added to 180-L fibreglass tanks supplied with both fresh water and seawater. One tank was prepared for each salinity treatment. Flow rates of fresh and salt water into common header mixing buckets were controlled by valves to achieve the desired salinities (±1 ppt) in the rearing tanks. The fish were gradually acclimated to the test salinities in a stepwise manner at a rate of 1–2 $ppt \cdot d^{-1}$. Salinity, temperature, dissolved oxygen, pH, and mortalities were recorded daily in each rearing tank. Water temperatures were kept constant (±1°C) using immersion heaters, and aeration was supplied to each tank to maintain dissolved oxygen levels near saturation. Dissolved oxygen levels ranged from 8.6 to 10.7 $mg \cdot L^{-1}$ due to differences in oxygen solubility with increasing salt content, but the minimum concentrations were not considered to be limiting to growth (Brett 1979). The fish were fed 4–5% of their total body weight daily by hand, using a diet of dry EWOS fry feed. The fish actively fed and there were no observable differences in swimming activity between the different salinity treatments.

Random samples of 20 steelhead trout and chinook salmon fry per treatment were measured for length and weight after the acclimation periods, and at 3-wk intervals throughout the experiments, to determine growth rates and feeding rations. Percent moisture content was determined from a sample of 10 chinook fry per treatment at each sampling period after oven drying at 80°C for 24 h. Blood was also collected from 10 fish per treatment at several sampling periods for the determination of hematocrit values and ion concentrations. After the fish was anesthetized, the caudal peduncle was severed and blood was collected from the dorsal aorta into heparinized capillary tubes. Hematocrits (percent red blood cells by volume) were determined after centrifugation at 10 000 rpm for 5 min. The separated plasma was withdrawn using Microcap® tubes and frozen at −20°C in 0.5-mL plastic vials prior to ion concentration analyses. Because of low plasma volumes in the smaller fish, it was necessary to pool samples in some cases to obtain sufficient volume for the ion analyses. Plasma chloride concentration ($[Cl^-]_{pl}$) determinations were made by coulometric titration (Haake Buchler Instruments digital chloridometer), while plasma sodium concentrations ($[Na^+]_{pl}$) were measured by flame photometry (Corning flame photometer model 410). Appropriate reference standards were run every 10 samples to ensure measurement accuracy. Water was collected from each rearing tank midway through the growth trials and analysed for Na^+, Cl^-, and Ca^{2+} for comparison with blood plasma values (Table 2).

At the end of each growth experiment, all remaining fish were measured for length and weight and 10 fish from each treatment were killed for hematocrit, $[Na^+]_{pl}$, and $[Cl^-]_{pl}$ determinations. Specific growth rates (expressed as percent change per day) were calculated for each treatment as $\ln (Wt_f \cdot Wt_i^{-1}) \cdot d^{-1} \cdot 100$ where Wt_i and Wt_f are the initial (after salinity acclimation) and final mean weights, respectively. Condition factors ($K = wt \cdot L^{-3} \cdot 100$; Ricker 1975) were also calculated from the length and weight data.

Respirometry Experiments

After the growth experiments were completed, oxygen consumption rates were measured for fish acclimated to the test salinities. The respirometers consisted of 1-L cylindrical glass chambers, 12 cm in diameter and 9 cm in height, and designed

TABLE 1. Experimental conditions for juvenile salmonid growth trials in different water salinities.

Parameter	Rainbow trout	Steelhead trout	Chinook salmon
Timing	Nov.–Dec. 1988	June–Sept. 1989	Feb.–June 1990
Duration	5 wk	12 wk	11 wk
Salinities (ppt)	0, 9[a], 18	0, 4, 8[a], 12, 16	0, 5, 10[a], 20, 28
Temperature (°C)	11	14	11.5
Photoperiod (h)	12 (constant)	14 (constant)	11–15 (natural)
No. of fish per tank	50	80	100
Initial mean weights (g)	1.0	0.4	1.0
Sampling frequency	5 wk	3 wk	3 wk

[a]Closest to isotonic value (i.e. 8–10 ppt).

TABLE 2. Chemical composition of water samples collected from the steelhead trout and chinook salmon fry rearing tanks (FW = freshwater; SW = seawater).

Salinity (ppt)	Chemical parameter			
	Na^+ (meq·L^{-1})	Cl^- (meq·L^{-1})	Ca^{2+} (meq·L^{-1})	pH
	Steelhead trout			
0 (FW)	3	<1	<1	6.0
4	71	63	1	7.1
8	146	120	1	7.3
12	189	170	2	7.4
16	213	240	3	7.5
28 (SW)	386	450	6	7.7
	Chinook salmon			
0	3	<1	<1	6.0
5	81	71	1	7.1
10	163	156	3	7.3
20	273	288	5	7.5
28	371	456	7	7.6

to sit on magnetic stir plates. Water from an aerated and temperature controlled reservoir was passed through the chambers using a Masterflex® pump. Thick-walled Nalgene® tubing was used to connect the chambers to dissolved oxygen probes and a four-way valve assembly which allowed the respirometer to operate in an open (flow-through) or closed mode. Circular water currents within each respirometer chamber were generated by magnetic stirrers and speeds were controlled by calibrating digital rpm readouts on the stir plates with velocities determined for each chamber. Dissolved oxygen levels in each chamber were monitored continuously using polarographic oxygen electrodes and a multichannel oxygen meter (Orbisphere model 2710).

Measurements of respiratory metabolism in fish can be influenced by several factors including water temperature and fish size and activity (Brett and Groves 1979). To determine the effect of salinity alone, concerted efforts were made to control all other variables and therefore minimize variation in test results. Depending on the species, two to five fish of similar size were placed into each respirometer through a ground-glass lid and the flow rate was adjusted to approximately 100 mL·min^{-1}. The fish were acclimated to the respirometers for 24 h and were not fed for 48 h prior to testing to ensure a postabsorptive digestive state (Brett 1964). To shield the fish from outside disturbances, the respirometers were covered with black plastic throughout acclimation and testing. After acclimation, the fish were given a 15-min swimming adjustment period before the respiration trial. In each trial, the swimming speed was set to 1 body length·s^{-1} to standardize the level of activity (Brett 1962). This mild swimming challenge was necessary to reduce the variation in oxygen consumption caused by spontaneous activity at swimming speeds less than 1 body length·s^{-1} (Brett and Glass 1973). The respirometers were then closed and the subsequent decline in water oxygen concentration (to the nearest 0.05 mg·L^{-1}) was monitored for 1 h, with values recorded every 5 min. After a trial, the fish were removed and measured for length and weight. The trials were conducted at approximately the same time each day to minimize diurnal variation in metabolism due to entrainment to a feeding schedule or photoperiod (Brett and Zala 1975). Water temperatures were kept similar to those used in the growth experiments (Table 1). Background oxygen consumption, due to bacteria, was measured by running blanks (i.e. no fish) throughout the experimental period. A correction was applied to the fish respiration measurements assuming a linear increase of bacterial oxygen consumption.

Water oxygen concentrations initially declined in a curvilinear fashion for about 10–15 min, apparently as a result of pressure changes associated with closing the system (Rombough 1988). Oxygen levels then decreased at a constant rate for about 30–45 min. Approximately 25% of the initial oxygen was consumed during the linear portion of the measurements. Oxygen consumption rates were estimated over the linear portion using regression analysis. Metabolic rates were determined by multiplying the regression slope of oxygen uptake by the respirometer volume and were expressed in terms of wet weight over a 1-h period (i.e. milligrams oxygen per kilogram per hour). Six replicate trials were conducted per salinity treatment for the steelhead trout and chinook salmon and three for the rainbow trout fry.

Preliminary trials with rainbow trout fry in fresh water showed that oxygen consumption increased linearly with increasing fish weight when expressed on a double logarithmic grid (slope = 0.77). This is a well-established relationship in studies on respiratory metabolism of fish (Brett and Groves 1979) and was used in the present study to validate the method of measuring oxygen consumption in the circular chambers.

Seawater Challenge Testing

At the end of the study, juvenile steelhead trout and chinook salmon (average weights = 7–10 g) were subjected to seawater challenge tests (Blackburn and Clarke 1987) to determine the effect of prior acclimation to the dilute salinities on subsequent adaptability to full-strength seawater. Random samples of 10–12 fish were transferred from the test salinities to 28–29 ppt (with the exception of the chinook 28 ppt treatment which had only four fish of small size remaining) and were sampled after 24 h of exposure for $[Na^+]_{pl}$ as described before. The criterion for proper seawater adaptation is the ability to maintain $[Na^+]_{pl}$ less than 170 meq·L^{-1} 1 d after transfer to seawater (Clarke et al. 1981).

Statistical Analysis

Data are presented as means ± standard error (SE) where appropriate. When analysis of variance indicated significant effects, Tukey's multiple comparison test (Steel and Torrie 1980) was used to identify significantly different treatment means ($P < 0.05$). All analyses were performed with the SYSTAT statistical program (Wilkinson 1988).

Results

Mortality

Mortality was less than 5% in all treatments except for chinook salmon fry reared in 28 ppt, which suffered a loss of 24 fish (Table 3). The majority of the mortalities were less than 5 cm in fork length and weighed less than 1.5 g.

Growth and Condition

Growth rates declined with increasing salinity for all three species (Fig. 1; Table 3). Rainbow trout fry growth decreased

TABLE 3. Mortality, specific growth rates, condition factors, and moisture content of juvenile rainbow and steelhead trout and chinook salmon reared in various salinities. Mean values followed by the same letter are not significantly different ($P < 0.05$) by Tukey's test.

Salinity treatment (ppt)	Percent mortality	Specific growth rate (% wt·d^{-1})	Final mean condition factor (± SE)	Final mean percent moisture content (± SE)
		Rainbow trout		
0	2	3.35	1.13 ± 0.01 a	
9	0	3.25	1.17 ± 0.01 a	
18	4	1.57	1.07 ± 0.01 b	
		Steelhead trout		
0	3	3.27	1.12 ± 0.01 a	
4	0	2.67	1.15 ± 0.01 b	
8	0	2.98	1.19 ± 0.01 c	
12	0	3.11	1.17 ± 0.01 bc	
16	0	2.75	1.17 ± 0.01 bc	
		Chinook salmon		
0	0	2.62	1.05 ± 0.01 a	78.0 ± 0.2 a
5	0	2.44	1.06 ± 0.01 a	78.1 ± 0.2 ab
10	0	2.32	1.06 ± 0.01 a	78.6 ± 0.2 ab
20	0	2.30	1.07 ± 0.01 a	79.1 ± 0.2 b
28	24	1.84	1.15 ± 0.01 b	81.4 ± 0.4 c

slightly from fresh water to isotonic salinity and decreased significantly in the hypertonic treatment. Steelhead trout fry showed a similar response, but final weights in 4 ppt were significantly lower than in fresh water (Fig. 2). Growth of chinook salmon fry was significantly higher in fresh water than all other treatments and showed a steady decline with increasing salinity to full-strength seawater. Specific growth rate values for chinook salmon fry in fresh water and seawater were similar to those reported by Clarke et al. (1981).

Condition factors of steelhead trout and chinook salmon fry did not differ significantly between salinity treatments for the first three sampling periods of the growth trials. Final condition factors varied among growth experiments, showing no consistent pattern between species (Table 3). Rainbow trout fry in fresh water and isotonic salinity had significantly higher final condition factors than the hypertonic treatment, but the condition factor for steelhead trout fry in fresh water was significantly lower than the other treatments. Condition factors for the chinook salmon fry were similar up to 20 ppt, but were significantly higher in 28 ppt; fish in full-strength seawater tended to be deeper bodied than the more slender body forms in the other treatments. The initial moisture content of the chinook salmon fry was 81% in all treatments and, as expected, declined with growth of the fry (Clarke et al. 1981). Differential growth rates between salinity treatments, however, resulted in size differences which masked the anticipated dehydration effect of salinity on moisture content (Table 3).

Hematocrit and Plasma Ion Concentrations

Hematocrit values for rainbow trout fry increased with salinity and were significantly higher in 18 ppt than in fresh water (Fig. 3). Hematocrit values for steelhead trout fry were also significantly higher in 16 ppt than in fresh water on day 63, but this pattern was not repeated at the other sampling times (Fig. 4). Hematocrit values for chinook salmon fry followed an opposite trend from the trout and decreased with increasing salinity, being significantly lower in 20 and 28 ppt than in fresh water on day 56 (Fig. 5). $[Na^+]_{pl}$ measured in rainbow trout did not differ significantly among treatments, while $[Cl^-]_{pl}$ was significantly higher in 18 ppt (Fig. 3). $[Na^+]_{pl}$ in steelhead trout fry tended to increase with salinity and was significantly higher in 12 and 16 ppt compared with fresh water on day 84. $[Cl^-]_{pl}$ did not differ significantly among treatments, although it tended to be higher at 12 and 16 ppt on days 42 and 63 (Fig. 4). $[Na^+]_{pl}$ and $[Cl^-]_{pl}$ in chinook salmon fry did not differ significantly among treatments at each sampling period (Fig. 5).

Metabolic Rates

The average metabolic rates of juvenile salmonids in fresh water measured in the present study ranged from 207 to 270 mg O_2 kg^{-1}·h^{-1}, depending on species and temperature, and were comparable with values obtained by Brett (1965) for juvenile sockeye salmon (*Oncorhynchus nerka*) of similar size and activity. Metabolic rates of all three species increased with salinity, being significantly higher in hypertonic treatments than in fresh water (Fig. 6). The magnitude of these changes in metabolic rate from fresh water to 16–20 ppt ranged from 25 to 30%. The following linear regression equations describe the relationship between metabolic rate and salinity, determined for the three species:

(1) Rainbow trout: $\ln \dot{M}O_2 = 0.080 \cdot \ln (S + 1) + 5.328$; $r^2 = 98.6\%$

(2) Steelhead trout: $\ln \dot{M}O_2 = 0.064 \cdot \ln (S + 1) + 5.581$; $r^2 = 75.2\%$

(3) Chinook salmon: $\ln \dot{M}O_2 = 0.087 \cdot \ln (S + 1) + 5.569$; $r^2 = 93.7\%$

where $\dot{M}O_2$ is metabolic rate (milligrams oxygen per kilogram

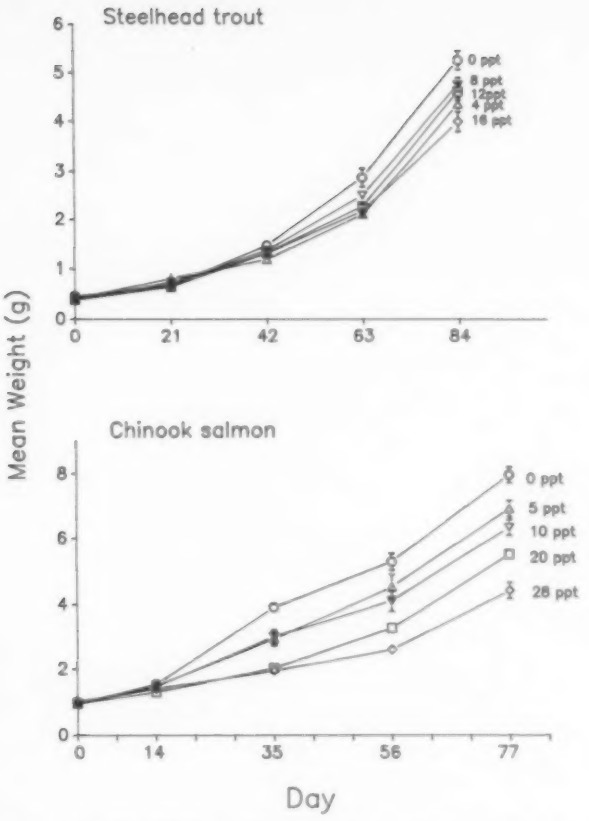

FIG. 1. Growth curves for steelhead trout and chinook salmon fry reared in five different salinities. Absence of standard error bars indicates that the SE was smaller than the symbol.

per hour) and S is salinity (parts per thousand). These equations provide good estimates of metabolic rates for the size of fish, water temperatures, activity level, and range of salinities used in this study. The increases in metabolic rate also showed significant inverse linear correlation to the observed decreases in growth (r^2 values: rainbow = 0.69, steelhead = 0.63, chinook = 0.89).

Seawater Challenge Tests

No mortalities were recorded during the 24-h seawater challenge tests. $[Na^+]_{pl}$ in juvenile steelhead trout and chinook salmon after 24 h of exposure to seawater were all below the 170 meq·L^{-1} threshold and did not differ significantly among treatments, with the exception of steelhead trout transferred from 4 ppt which averaged 177.4 ± 3.6 meq·L^{-1} (Fig. 7). Acclimation to dilute salinities therefore had little influence on the seawater adaptability of juvenile steelhead trout and chinook salmon in the present study.

Discussion

The mortality data indicate that the small fry stages (approximately 0.5–1.0 g) of these species can adapt to salinities of up to 20 ppt when a gradual acclimation procedure is followed. Previous studies on seawater adaptation have also

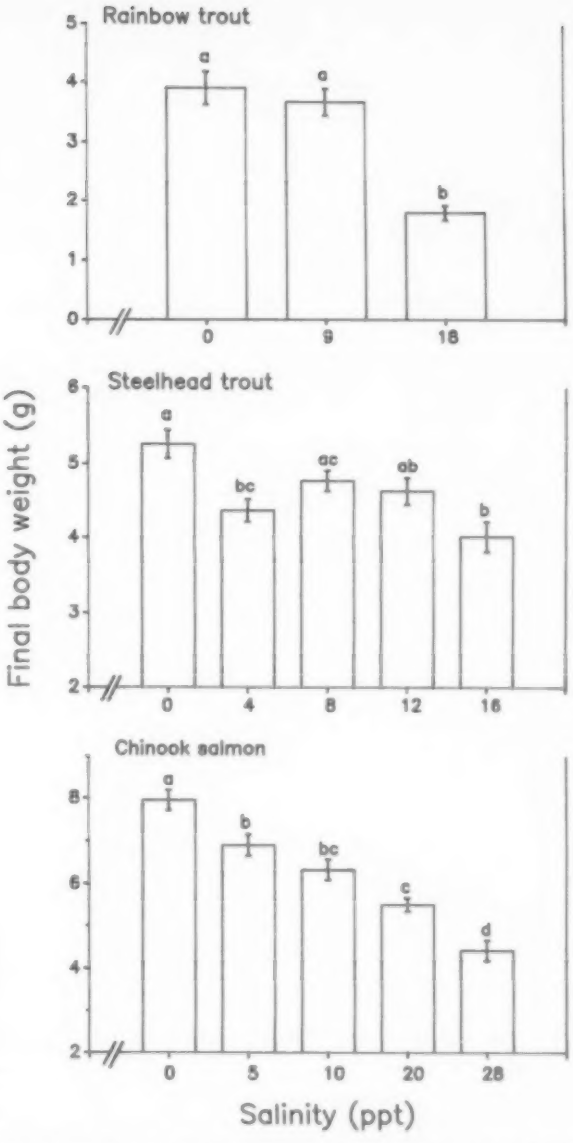

FIG. 2. Effect of salinity on growth of juvenile rainbow and steelhead trout and chinook salmon (starting weights = 1.0, 0.4, and 1.0 g, respectively). Means (± 1 SE) with a common letter are not significantly different by Tukey's test.

shown increased survival of 1-g chinook salmon fry following gradual transition to seawater (Wagner et al. 1969; Kepshire and McNeil 1972) and 20 ppt salinity to be an upper threshold limit for the survival of slightly larger (10–30 g) rainbow trout (Landless 1976; Eddy and Bath 1979; Jackson 1981; Johnsson and Clarke 1988) and Atlantic salmon (*Salmo salar*) parr (Saunders and Henderson 1969a).

The measured values for both $[Na^+]_{pl}$ and $[Cl^-]_{pl}$ in fresh water are in good agreement with normal values for all three species (Conte and Wagner 1965; Wagner et al. 1969; Hille 1982). $[Na^+]_{pl}$ and $[Cl^-]_{pl}$ in rainbow and steelhead trout rose slightly when reared in salinities above the isotonic level, while

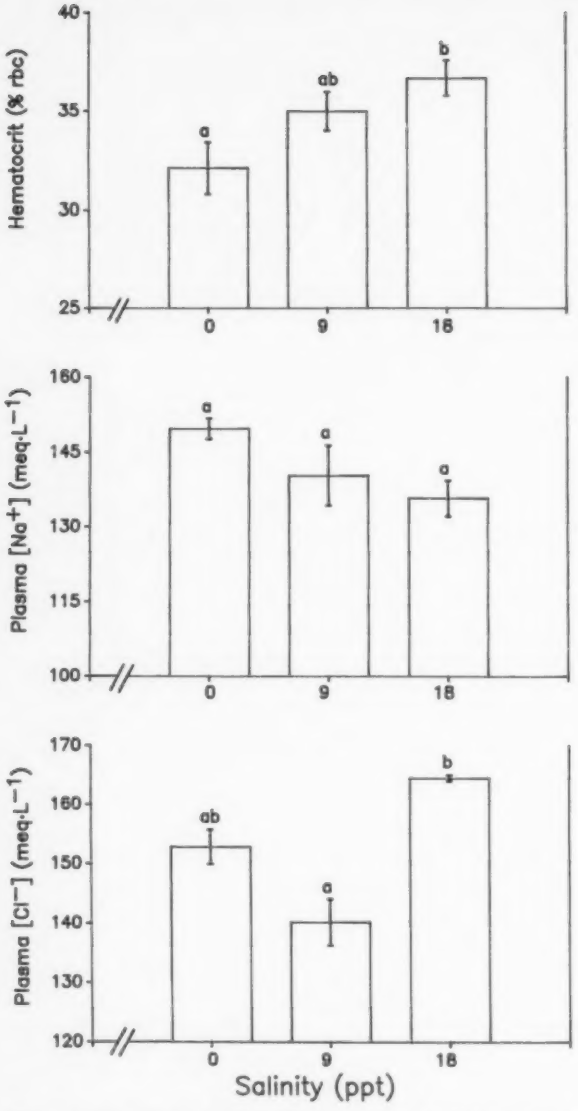

FIG. 3. Mean hematocrit and $[Na^+]_{pl}$ and $[Cl^-]_{pl}$ for rainbow trout fry after 5 wk in three different salinities. Means (± 1 SE) with a common letter are not significantly different by Tukey's test.

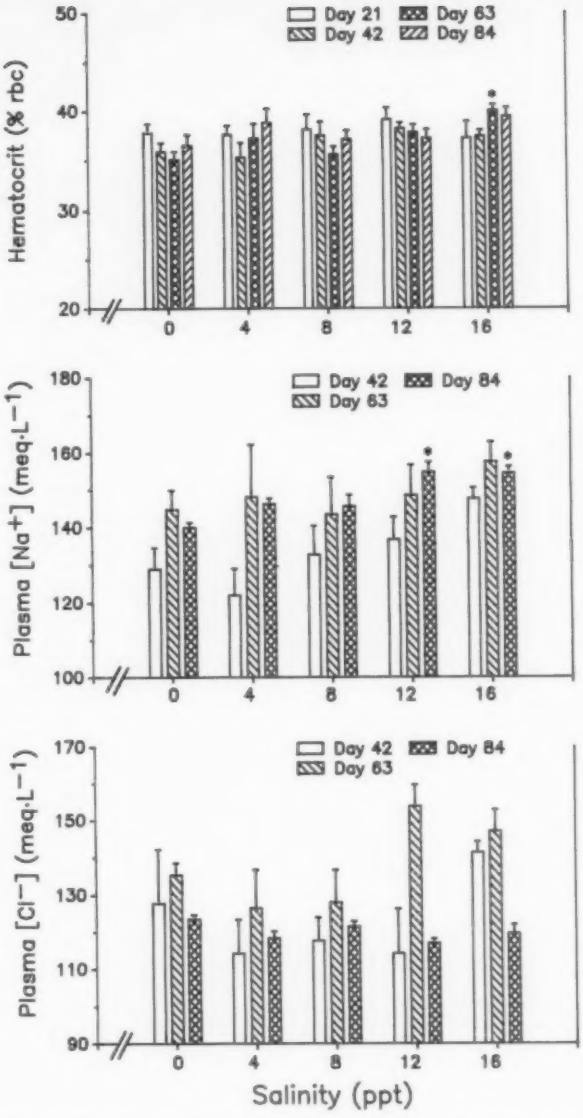

FIG. 4. Mean hematocrit and $[Na^+]_{pl}$ and $[Cl^-]_{pl}$ ($+1$ SE) for steelhead trout fry reared in five different salinities. Asterisks denote salinity treatment means which are significantly different from the freshwater value.

plasma ion concentrations in chinook salmon fry did not change across all salinities (the ionic status of mortalities in 28 ppt could not be determined). Despite these slight increases (8–10%) in the trout, plasma ion concentrations for all three species were maintained within acceptable levels (i.e. $[Na^+]_{pl} < 180$ meq·L^{-1}, $[Cl^-]_{pl} < 160$ meq·L^{-1}, Eddy and Bath 1979; Johnston and Cheverie 1985; Stagg et al. 1989), indicating that the fish were regulating blood ions competently against concentration gradients (Table 2). It is likely, therefore, that the observed growth and metabolic rates reflect steady state in fish with respect to ionic status. The increase in Ca^{2+} content of the external media at higher salinities may have assisted in regulating the passive movements of Na^+ and Cl^-, as Ca^{2+} is known to alter the permeability of gill membranes to these ions (Eddy 1975). The variable responses of hematocrit to salinity observed in the present study have been reported previously (Leatherland and McKeown 1974; Zeitoun et al. 1974; Bath and Eddy 1979) and may be explained by differential changes in red cell and plasma volumes which appear to be species specific.

The results of the present laboratory experiment indicated that salinity had a negative effect on the growth of all three species, particularly at salinities above the isotonic level. These findings are in agreement with Shaw et al. (1975), Clarke et al. (1981), McKay and Gjerde (1985), and McCormick et al. (1989c), who also found that isotonic salinity was not the point

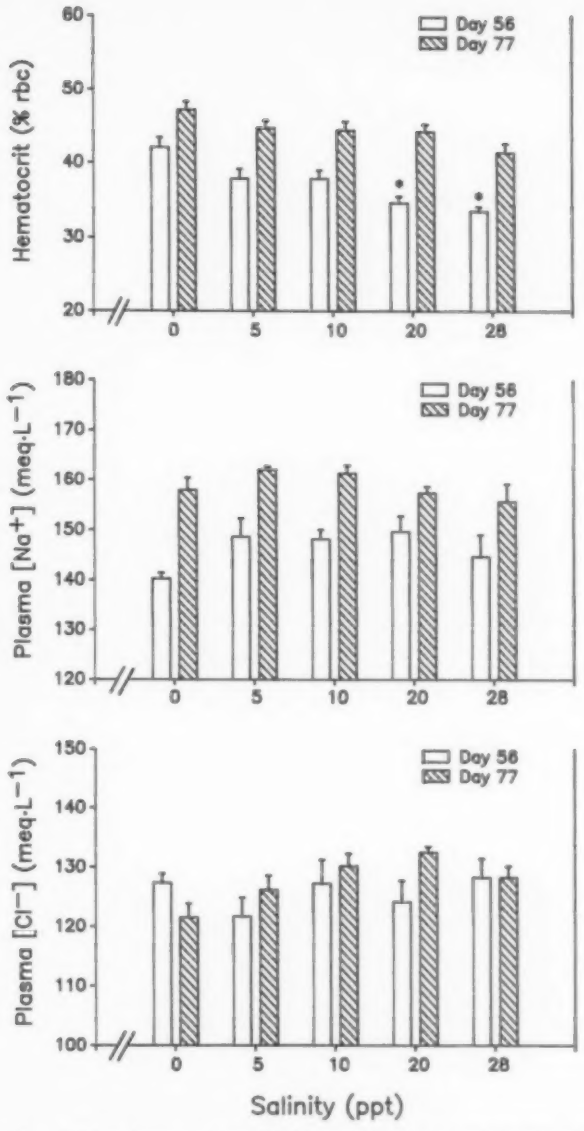

FIG. 5. Mean hematocrit and $[Na^+]_{pl}$ and $[Cl^-]_{pl}$ (+1 SE) for chinook salmon fry reared in five different salinities. Asterisks denote salinity treatment means which are significantly different from the freshwater value.

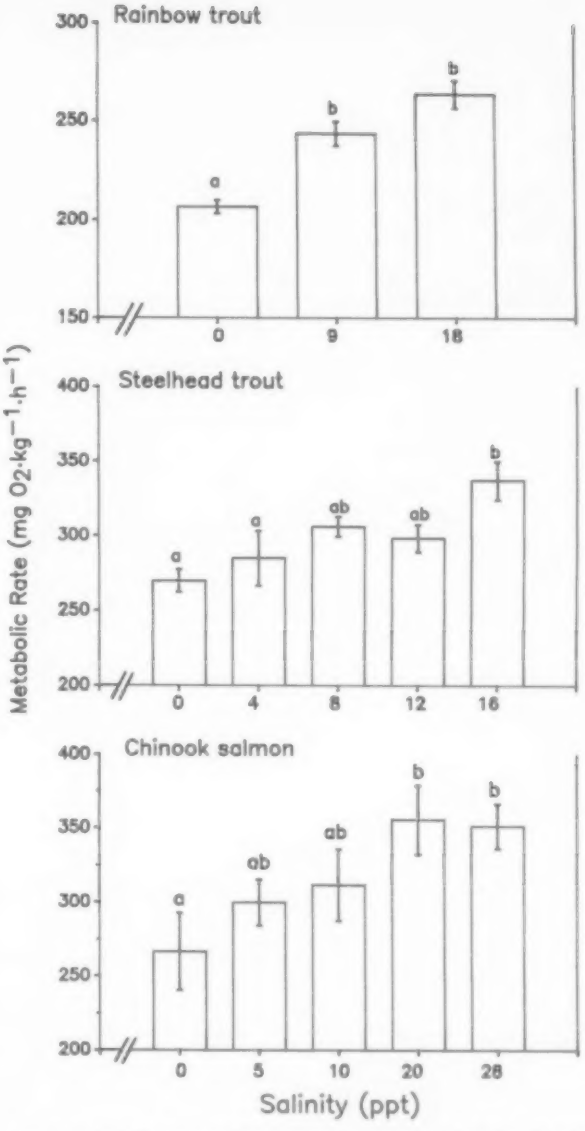

FIG. 6. Metabolic rates of juvenile rainbow and steelhead trout and chinook salmon in different salinities. Means (± 1 SE) with a common letter are not significantly different by Tukey's test.

of maximal growth in juvenile salmonids. The works of Canagaratnam (1959) and Otto (1971) with coho salmon presmolts are widely quoted in the literature as studies which support the notion of an optimum growth response in an isotonic environment. Subsequent experiments, however, have revealed conflicting results. Canagaratnam (1959) used small aquaria with static water to study the growth response of sockeye and chum (*Oncorhynchus keta*) as well as coho salmon fry to different salinities. This resulted in low overall specific growth rates (1.25%·d^{-1} for coho fry in fresh water) and very high mortalities in the sockeye and chum fry tests. It is possible, therefore, that confinement stress affected the study results. In the experiments conducted by Otto (1971), growth rates of coho salmon fry were actually higher in fresh water from June to September and increased in 10 ppt salinity only from October to February. This shift in salinity for optimal growth during the fall is consistent with other studies on the behaviour and physiology of coho salmon presmolts. Otto and McInerney (1970) found that the preferred salinity for the same stock of coho salmon fry changed during October and November to 10 ppt, corresponding with the growth results. Conte et al. (1966) also found that juvenile coho salmon were able to tolerate full-strength seawater in October, 6 mo prior to the usual seawater migration period. Lasserre et al. (1978) further showed that the activity of gill Na^+,K^+-ATPase, the enzyme located in chloride cells

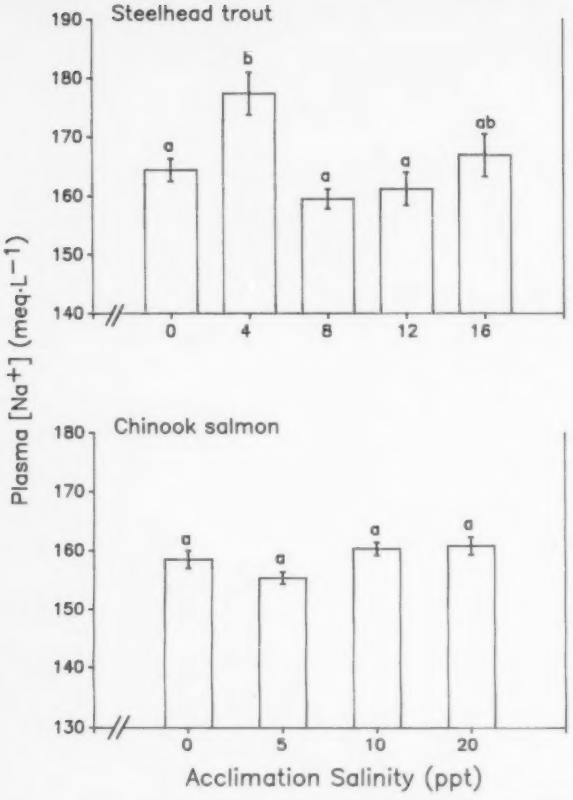

FIG. 7. $[Na^+]_{pi}$ of juvenile steelhead trout and chinook salmon following a 24-h seawater challenge test. Means (± 1 SE) with a common letter are not significantly different by Tukey's test.

which is indicative of ion excretion capacity (see review by Folmar and Dickhoff 1980), increased during mid-October in coho salmon underyearlings in fresh water. This resulted in improved growth and survival of the fish when they were transferred to seawater netpens during autumn compared with other times of the year (Harache et al. 1980). These results are all consistent with the observation that coho salmon are sometimes found in estuaries as underyearlings (Tschaplinski 1987) and have therefore evolved physiological mechanisms to optimize growth in changing environments. A similar rise in gill Na^+, K^+-ATPase activity and estuarine residence during autumn has also been reported for Atlantic salmon parr (Langdon and Thorpe 1985; Cunjak et al. 1989). Determinations of the optimal salinity for growth in salmonids must therefore consider the influences of species-specific differences, developmental stage, size, and season (Saunders and Henderson 1969b; McCormick and Naiman 1984; McCormick et al. 1989c).

The highest growth rates of juvenile salmonids in the present study were associated with fresh water, which is also the natural habitat for these species at this life stage. Growth of landlocked rainbow trout fry decreased 53% from fresh water to the hypertonic salinity of 18 ppt, while the growth of anadromous steelhead trout and chinook salmon fry declined only 16 and 12%, respectively, over a similar range. These data indicate that even at the fry stage, anadromous steelhead trout and chinook salmon adapt and grow better at hypertonic salinities than the freshwater resident rainbow trout. Although not examined in the present study, it is possible that the steelhead trout and chinook salmon fry possessed a greater number of, or more active, ion-excreting chloride cells on the gill, which are known to be poorly developed and fewer in number in strictly freshwater fish than in anadromous or marine species (Folmar and Dickhoff 1980; Burton and Idler 1984).

Contrary to our initial hypothesis, we found that oxygen consumption rates for all three species of salmonids were not lowest in isotonic salinity; they were lowest in fresh water. The higher metabolic rates at higher water salinities apparently reflected a significant energetic cost, as growth rates declined with increasing salinity, and correlated very well with the changes in oxygen consumption. Assuming that these increased energy demands were related solely to ionic and osmotic regulation (= ion-osmotic regulation), the metabolic rate data suggested that the energetic cost of ion-osmotic regulation for fry of these salmonid species was lowest in fresh water and increased to 12–18% in isotonic salinity.

It is difficult to reconcile the differences in the metabolic responses to changes in salinity found in the present study with those previously reported. In other studies, total metabolic rates measured in acclimated fish have either increased, decreased, or remained unchanged as salinity increased. From the recent literature (Table 4), we have compiled and categorized five patterns of metabolic responses of teleost fish to altered salinities, some of which are similar to those proposed by Nordlie (1978). These patterns include the following: (I) metabolic rate does not change over a wide range of salinities, (II) metabolic rate is minimum in isotonic salinity and increases at lower and higher salinities, (III) metabolic rates are minimum in fresh water and increase at higher salinities, (IV) metabolic rates are highest in fresh water and decrease to isotonic (higher salinities are not tolerated), and (V) metabolic rates are lowest in seawater and increase in lower salinities.

Life habits will, to a certain extent, determine the type of metabolic response to changes in salinity (Table 4). Euryhaline fish which move freely through waters of varying salinity generally demonstrate a type I response, i.e. metabolic rates do not change. Euryhaline forms which are found in fresh water as adults or in estuaries as juveniles tend to illustrate a type II metabolic response (minimum in isotonic salinity) whereas fish which are found as juveniles in fresh water or in estuaries as adults display a type III response pattern (metabolism increases with salinity). Stenohaline freshwater fish show a type IV response, which probably reflects a depression of metabolic rate due to stress. Finally, fish species which are found primarily in marine habitats illustrate a type V response, which is in accordance with Prosser's (1973) general hypothesis that for euryhaline marine species, metabolic rates increase at reduced salinities. It is also apparent from this summary of metabolic patterns that juveniles frequently respond differently than do adults of the same species (e.g. *O. tshawytscha, Ambassis interrupta*) and, in general, are more influenced by salinity variations than are the larger adults, a point also recognized by Nordlie (1978). It is evident from the studies cited in Table 4, therefore, that the lowest metabolic rates in response to salinity are associated with the environments in which the species are most commonly found, and presumably most physiologically adapted, for a particular life stage. The strong correlation between optimum growth and metabolic rates in response to salinity observed in the present study supports this hypothesis.

TABLE 4. Metabolic response patterns of teleost fish to changes in environmental salinity, compiled from recent literature.

Metabolic response	Species and life stage	Habits[a]	Reference
I. No significant change in metabolic rate over a wide salinity range	*Oncorhynchus tshawytscha* (chinook salmon), subadult	Anad-Est/Mar	Bullivant 1961
	Kuhlia sandvicensis (aholehole), adult	Eur-Mar	Muir and Niimi 1972
	Aphanius dispar (cyprinodont), juvenile/adult	Eur-Mar	Shakhauge and Lotan 1974
	Gillichthys mirabilis (gobie), adult	Eur-Mar	Courtois 1976
	Ambassis interrupta, adult	Eur-Est	Nordlie 1978
	Fundulus heteroclitus (mummichog), adult	Eur-Bra	Nead and Buttner 1987
	Girella nigricans (percoid), adult	Eur-Mar	Fang 1982
II. Metabolic rate is minimum in isotonic salinity and increases at lower and higher salinities	*Oncorhynchus mykiss* (rainbow trout), adult	Eur-Fw	Rao 1968
	Tilapia nilotica (tilapia), adult	Eur-Fw	Farmer and Beamish 1969
	Brevoortia tyrannus (Atlantic menhaden), juvenile	Eur-Est	Hettler 1976
	Ambassis interrupta, juvenile	Eur-Est	Nordlie 1978
	Mugil curema (white mullet), juvenile	Eur-Est	Fanta-Feofiloff et al. 1986
	Oreochromis niloticus (tilapia), juvenile	Eur-Est	DeSilva et al. 1986
	Oreochromis mossambicus (tilapia), adult	Eur-Fw	Febry and Lutz 1987
III. Metabolic rate is lowest in fresh water and increases with salinity	*Platichthys stellatus* (starry flounder), juvenile/adult	Eur-Est	Hickman 1959
	Mugil cephalus (striped mullet), adult	Eur-Est/Mar	Nordlie and Leffler 1975
	Cyprinodon salinus (pupfish), adult	Eur-Bra	Stuenkel and Hillyard 1981
	Oncorhynchus kisutch (coho salmon), juvenile	Anad-Fw	Nagibina 1983
	Leiostomus xanthurus (spot), juvenile	Eur-Est	Moser and Hettler 1989
	Oncorhynchus mykiss (rainbow and steelhead trout), juveniles *Oncorhynchus tshawytscha* (chinook salmon), juveniles	Anad-Fw	This study
IV. Metabolic rate is highest in fresh water and decreases to isotonic (higher salinities are not tolerated)	*Ctenopharyngodon idella* (grass carp), juvenile	Sten-Fw	Maceina et al. 1980
	Ictalurus nebulosus and *I. punctatus* (catfish), adult	Sten-Fw	Furspan et al. 1984
V. Metabolic rate is minimum in seawater and increases in lower salinities	*Mugil cephalus* (striped mullet), adult	Eur-Mar	Marais 1978
	Mugil macrolepis (mullet), adult	Eur-Mar	Mathew 1978
	Cyprinodon variegatus (sheepshead minnow), juvenile/adult	Eur-Bra	Barton and Barton 1987

[a]Anad, anadromous; Est, estuarine; Mar, marine; Eur, euryhaline; Bra, brackish; Fw, fresh water; Sten, stenohaline.

Given that the mechanisms of ion-osmotic regulation are similar among fish species (Evans 1984), the variety of metabolic responses to different salinities depending on species, habits, and life stages suggests that these responses are being influenced by physiological processes in addition to the energy required for ion-osmotic regulation (Febry and Lutz 1987). McCormick et al. (1989a), for example, have recently reported that the changes in the metabolic capacity of isolated gill and kidney tissues of Atlantic salmon in different salinities did not account for the magnitude of changes seen in whole-body metabolism. During acclimation to different salinities, hormonal changes occur in fish which, in addition to their role in

ionic regulation, may also have secondary effects on metabolism not directly related to ion exchange between blood and water. Cortisol, for example, is known to promote branchial ion excretion by stimulating chloride cell proliferation, differentiation, and secretory activity (Foskett et al. 1983; McCormick et al. 1989b; Madsen 1990a, 1990b) and shows peaks during the parr–smolt transformation of juvenile salmonids in fresh water (Specker and Schreck 1982). This hormone has also shown long-term increases in juvenile salmonids after entry into seawater (Redding et al. 1984; Young et al. 1989; Avella et al. 1990) and has been associated with increases in metabolic rate (Chan and Woo 1978; Barton and Schreck 1987). Therefore, the increase in metabolic rates observed for juvenile salmonids in the present study may also have been related to a more general metabolic response of the test species to increasing salinity, as opposed to a strictly energetic cost of ion-osmoregulation. The confounding effects of salinity changes on overall metabolism make it difficult to distinguish metabolic costs exclusively associated with ion-osmotic regulation. The estimates provided previously, therefore, probably overestimate the energetic cost of ion-osmotic regulation. More detailed and quantitative descriptions of the physiological responses of fish to changes in water salinity that account for differences in species, habits, and life stages are necessary to accurately assess the energetic cost of ionic regulation. For example, comparison of gill oxygen consumption with total body metabolism in vivo may assist in separating energetic activities associated with ionic regulation from other metabolically active processes involved with adjustments to alterations in water salinity.

Acclimation to dilute salinities did not improve the osmoregulatory performance of juvenile steelhead trout and chinook salmon challenged with an acute (24-h) exposure to full-strength seawater. Similar results were obtained by Clarke et al. (1981) for coho and chinook salmon reared at 10°C. The use of this technique in a hatchery prior to seawater transfer is therefore probably not necessary, once the fish have reached the critical size and developmental state for smolting. Freshwater rearing temperature, fish size, and time of transfer have been shown to be the most important factors influencing adaptability and growth in seawater for juvenile fall chinook salmon (Clarke and Shelbourn 1985). The absence of mortalities during the 24-h seawater challenge tests were unexpected for the 7- to 9-g steelhead trout, whose critical size for seawater adaptation is generally accepted to be about 35 g (Conte and Wagner 1965; Clarke 1982). A possible explanation for these findings may be that a constant spring photoperiod (14 h) was used throughout the steelhead experiment, and this has been shown to accelerate seawater adaptation in juvenile steelhead trout (Johnsson and Clarke 1988).

In summary, growth and metabolic rates of rainbow trout, steelhead trout, and chinook salmon fry were optimum in fresh water, their natural habitat at this life stage. Isotonic salinity did not provide metabolic or growth advantages in the present study, despite the hypothesis that it would provide the lowest energetic demands for ion-osmoregulation. Comparison with other studies indicated that optimal salinities for growth and metabolic rates were influenced by species, life stage, and season. Although the oxygen consumption data suggested that the energetic cost of ion-osmotic regulation increased with salinity, attempts to quantify this cost were probably affected by other metabolic processes which respond to changes in salinity.

Acknowledgements

Financial support for this research was provided by an NSERC operating grant to G.K.I. and a B.C. Science Council GREAT award to J.D.M. Facilities were supplied by E.V.S. Consultants, North Vancouver, and the Pacific Biological Station, Nanaimo, and the staffs of these institutions are thanked for their assistance.

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Size-Dependent Spatial Distribution of Hake (*Merluccius capensis* and *Merluccius paradoxus*) in Namibian Waters

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Data on hake (*Merluccius capensis* and *Merluccius paradoxus*) abundance along the Namibian coast showed both species to have strongly aggregated spatial distributions. In addition, average fish size increased with depth, suggesting that the formation of hake aggregation is based on size-dependent shoaling behaviour. Hake spacing was size dependent with areal fish density and biomass being scaled to the -5 and -2 power, respectively, of fish length. The equations describing the size dependence of the areal fish density were log density (fish·m^{-2}) = 5.06-4.91 log size (cm) for *M. capensis* and log density (fish·m^{-2}) = 5.28-4.74 log size (cm) for (*M. paradoxus*). The maximum areal densities, presumably corresponding to those within the shoals, exceeded those described by the equations above by 20- and 13-fold for *M. capensis* and *M. paradoxus*, respectively. The size dependence of hake spacing implies that fishing pressure targeting aggregates of small hake (i.e. shallow shoals) should have a disproportionate effect on fish mortality and recruitment compared with similar fishing pressure targeting aggregates of larger hake (i.e. deep shoals).

Des données sur l'abondance du merlu (*Merluccius capensis* et *Merluccius paradoxus*) dans les eaux côtières namibiennes révèlent que ces deux espèces ont une forte tendance à se regrouper en bancs. De plus, la taille moyenne du merlu augmente en fonction de la profondeur; ce fait porte à croire que le regroupement est basé sur un comportement lié à la taille. L'espacement entre les merlus dépendait de la taille: ainsi, la densité par unité de surface des poissons et de la biomasse peut être exprimée en termes de la longueur du poisson à la puissance -5 et -2, respectivement. Les équations décrivant la dépendance de la densité par unité de surface des poissons en fonction de la longueur sont les suivantes : log densité (poissons·m^{-2}) = 5,06 - 4,91 log taille (cm) dans le cas de *M. capensis* et log densité (poissons·m^{-2}) = 5,28 - 4,74 log taille (cm) dans le cas de *M. paradoxus*. Les densités maximums, qui correspondent vraisemblablement à celles des bancs de merlu, sont supérieures à celles décrites par les équations susmentionnées par 20 et 13 fois, respectivement, dans le cas de *M. capensis* et *M. paradoxus*. Le rawpport entre l'espacement des merlus et la taille signifie que la pression par pêche exercée sur des concentrations de petits merlus (c'est-à-dire les bancs à faible profondeur) devrait avoir une incidence disproportionnée sur le taux de mortalité et le recrutement du poisson par rapport à une pression par pêche semblable exercée sur des concentrations de gros merlus (c'est-à-dire les bancs à grande profondeur).

Received March 12, 1990
Accepted May 9, 1991
(JA504)

Reçu le 12 mars 1990
Accepté le 9 mai 1991

The spatial distribution of fish is an important component of fish behaviour that has significant implications for the exploitation of fish populations (Cushing 1976; Clark and Mangel 1979). Aggregated spatial distributions (i.e. fish shoals; cf. Pitcher 1983) appear to be the rule in fish populations (Radakov 1973). Fish aggregates tend to be highly structured because fish spacing within the shoals is size dependent (e.g. Serebrov 1976; Pitcher and Partridge 1979; Pitcher et al. 1985, 1986; Rose and Leggett 1988). Moreover, the size and composition of at least pelagic shoaling species appear to be constant irrespective of population size, which has a greater effect on the abundance of shoals (e.g. Murphy 1977; Csirke 1988).

Assessment and production models for demersal fish stocks often assume a uniform or random fish distribution. However, this assumption may be invalid for some demersal species which have shown changes in catchability over time (Angelsen and Olsen 1987; Crecco and Overholtz 1990; Rose and Leggett 1991). These studies suggests that aggregation in space may be the norm, rather than the exception, for demersal fish species (Rose and Leggett 1991). There is evidence that the Cape hakes (*Merluccius capensis* and *Merluccius paradoxus*), the more important commercial fish of the Benguela upwelling system (Newman 1977), exhibit aggregated spatial distributions because (1) the demersal fishery is virtually monospecific (Gordoa 1989), (2) there is high spatial variance in catches (Gordoa 1989), and (3) there is a gradation in fish size with increasing depth (Macpherson and Duarte 1991).

In this study, we examine catch data collected during a 3-yr study of *M. capensis* and *M. paradoxus* in the Benguela upwelling system to test the hypotheses that (1) the spatial distribution of these demersal species is aggregated (nonrandom) and (2) the density of fish within the aggregates is size dependent. We then examine the implications of our results for fishing mortality for these stocks.

Methods

The catch data used in this study were collected during five 20-d cruises from July 1985 to January 1989 along the conti-

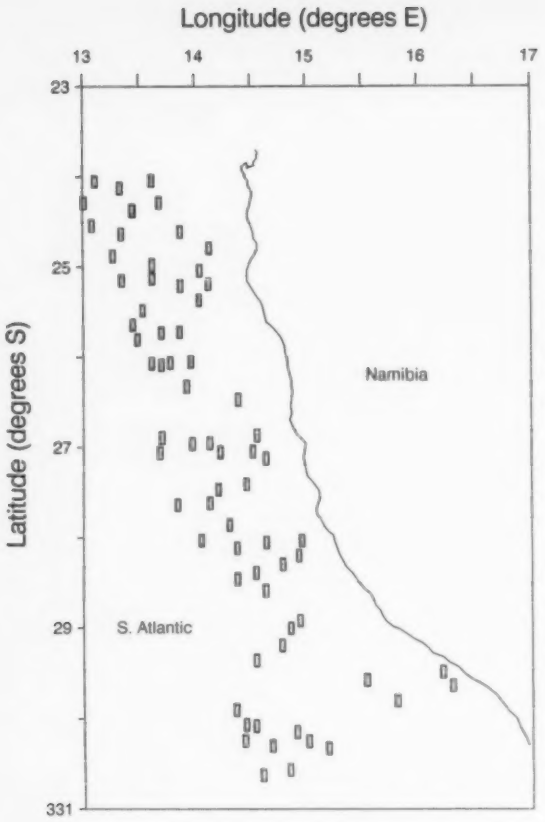

FIG. 1. Map of the studied area and the location of the sampling stations.

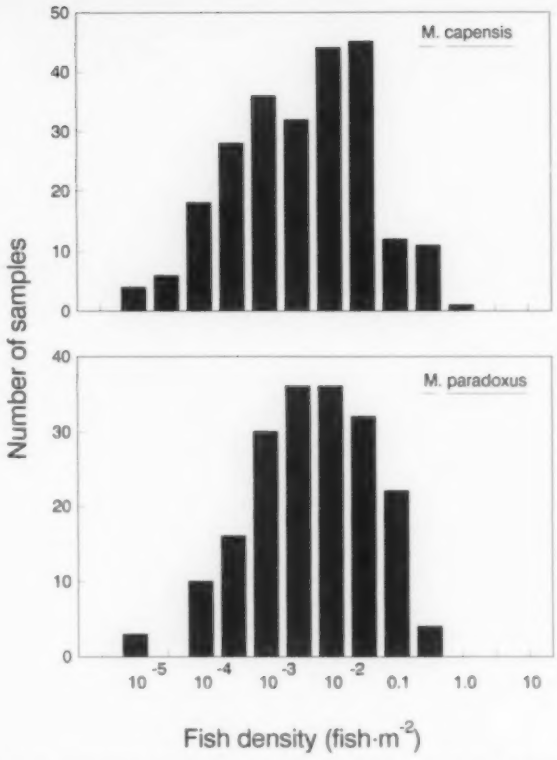

FIG. 2. Frequency distribution of fish catches (as areal density) for *M. capensis* and *M. paradoxus*.

nental shelf (100- to 500-m isobaths) of the Benguela upwelling region (95 000 km^2; Fig. 1). Upwelling-derived primary production in this region supports one of the world's largest fisheries (Cushing 1969). The total catch of hake species averages 400 000 tons·yr^{-1}, which represents about 90% of the catch of all demersal species (Newman 1977).

Fishing stations were located within the studied area using a stratified random design (Macpherson et al. 1985) (Fig. 1). Approximately 70 stations were sampled from a commercial stern trawler using bottom nets (two warps, headlines > 20 m, and heights between 2 and 4 m, 20-mm mesh size) on each cruise. Each set was 30 min at 3 knots, thereby sweeping 1.5 nautical miles. All fishing was conducted during daytime to avoid possible vertical migration off bottom during the night (Gordoa and Mapherson 1991). For each trawl set, the total number and weight of fish collected were determined for each species, and all fish collected (or a subsample of 250 when more than 300 were collected) were measured to calculate the average size (S, centimetres) of each species.

The spatial distribution of each species was characterized by examining the frequency distribution of catches (as the areal fish density) relative to the size of the fish caught. We note that our density estimates are minimum estimates of the "true" fish density within hake shoals because (1) catchabilities were almost certainly less than 100% and (2) since the sampling stations were randomly located, the proportion of the trawl length running over fish shoals was variable, thereby underestimating the "true" density when aggregation size was less than the scale of sampling (1.5 nautical miles).

As a consequence, regressions of areal fish density on fish size will tend to underestimate the density within shoals of fish of a particular size. Hence, we used the upper limit to the density estimates, represented by the upper band of the 95% confidence limits to the density estimates, to estimate the average "true" fish density within hake shoals. We assumed that the maximum observed densities corresponded to trawls wherein the swept areas were fully occupied by hake aggregates. This maximizing procedure influences the intercept but not the slope of the fitted regression equation. Consequently, our conclusions on the fisheries implications of the observed patterns, which are derived from the slope of the equation describing the size-dependent maximum density, are relatively independent of error in estimating maximum density.

We also estimated the average spacing of fish within hake shoals from the maximum density estimates. This was calculated as the equivalent diameter (D, metres) of the per capita space (G, square metres per fish, i.e. density^{-1}), derived from the expression (cf. Duarte et al. 1987)

$$(1) \quad D = (4 \times G/\pi)1/2.$$

The calculation assumes a two-dimensional distribution, which is an adequate description of the daytime distribution of both hake species (Gordoa and Macpherson 1991).

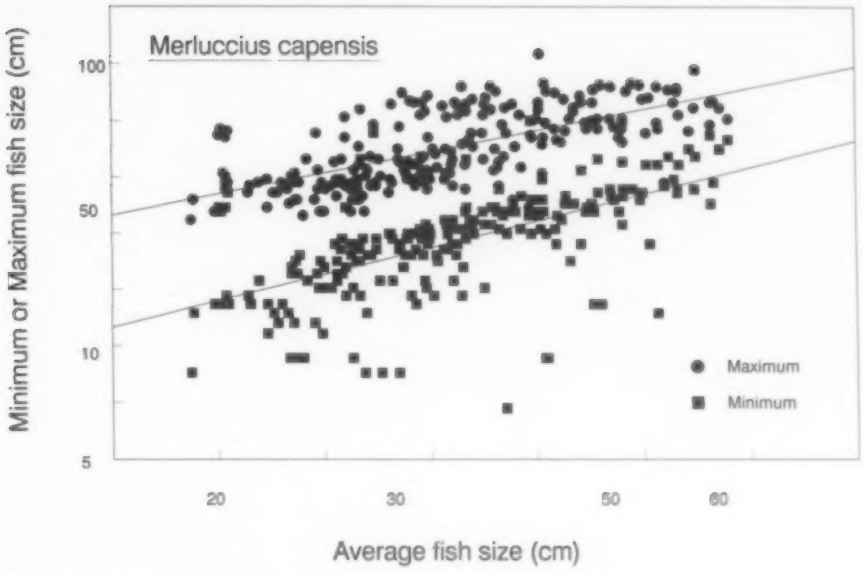

FIG. 3. Relationship between average fish length and the minimum and maximum fish length for *M. capensis*. Solid lines represent the fitted regression equations log minimum size (cm) = −0.38 + 1.14 log mean size (cm) (R^2 = 0.75) and log maximum size (cm) = 0.55 + 0.75 log mean size (cm) (R^2 = 0.58).

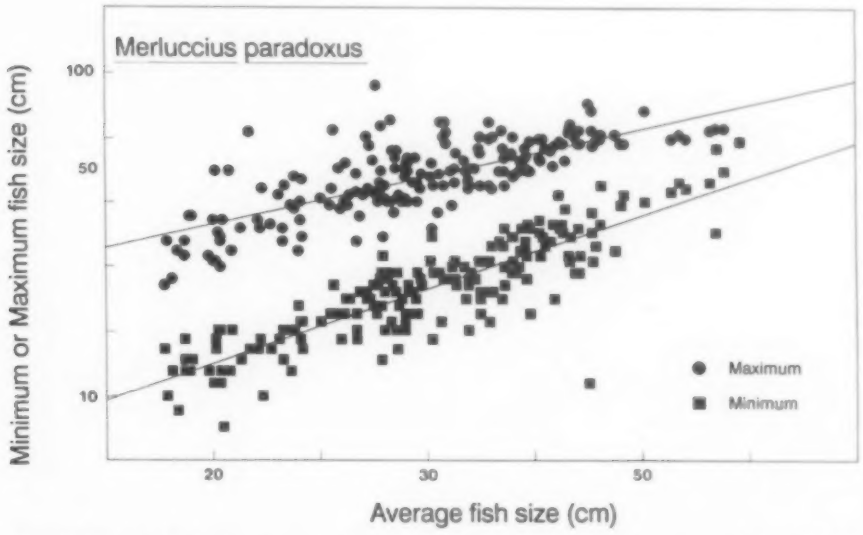

FIG. 4. Relationship between average fish length and the minimum and maximum fish length for *M. paradoxus*. Solid lines represent the fitted regression equations log minimum size (cm) = −0.19 + 1.03 log mean size (cm) (R^2 = 0.48) and log maximum size (cm) = 0.62 + 0.71 log mean size (cm) (R^2 = 0.50).

Results and Discussion

The distributions of fish catches were best represented by highly skewed (skewness 4.82 and 2.7 for *M. capensis* and *M. paradoxus*, respectively) log-normal distributions (P>0.10; Fig. 2), indicating strongly aggregated fish distributions. Several lines of evidence suggested that hake were segregated by size: (1) the positive relationships of average fish size to both maximum and minimum fish size in each set found for both hake species (Fig. 3 and 4), (2) the small variability of fish length within sets (average ± SE) coefficient of variation of fish length 16 ± 0.5 and 16 ± 0.4% for *M. capensis* and

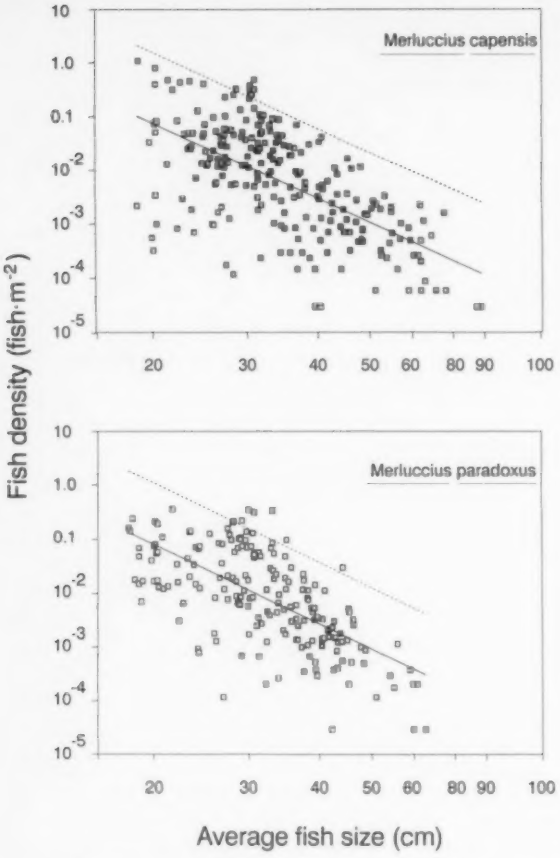

FIG. 5. Relationship between areal fish density and fish size for *M. capensis* and *M. paradoxus*. Solid line represents the fitted least squares regression equations (Eq. 2 and 3), and broken lines represent the upper band of the 95% confidence limits to the density estimates (Eq. 4 and 5).

M. paradoxus, respectively), and (3) fish size range that did not increase ($P > 0.10$) with increasing average fish size. These results are consistent with reports that hake size in Namibian waters increases, on average, with depth (Macpherson and Duarte 1991). These results suggest that the formation of hake aggregation is based also on size-dependent shoaling behaviour, as demonstrated for several species of pelagic fish (Freon 1984; Csirke 1988; Pitcher et al. 1985, 1986).

The areal fish density decreased with increasing average fish size (Fig. 5) as described by the equations

(2) log density (fish·m^{-2}) = 5.06-4.74 log size (cm)
$R^2 = 0.38$, $SE_{slope} = 0.38$, $SE_{estimate} = 0.79$, $P < 0.001$ (*M. capensis*)

and

(3) log density (fish·m^{-2}) = 5.28-4.91 log size (cm)
$R^2 = 0.42$, $SE_{slope} = 0.41$, $SE_{estimate} = 0.69$, $P < 0.001$ (*M. paradoxus*)

which indicate that the areal fish density within hake shoals is scaled to about length^{-5}. The coefficients and precision of the equations describing the relationship between areal fish density and average fish size (Eq. 2 and 3) did not differ significantly between species (*t*-test, $P > 0.05$).

We described the size-dependent upper limit to the density of hake populations, which should be closer to that within aggregations, by equations parallel to Eq. 2 and 3 through the upper band of the 95% confidence limits to the density estimates (Fig. 5):

(4) log density max. (fish·m^{-2}) = 6.35-4.91 log size (cm) (*M. capensis*)

and

(5) log density max. (fish·m^{-2}) = 6.40-4.74 log size (cm) (*M. paradoxus*).

These equations imply that the average distance between neighbour fish (cf. Eq. 1) changes as

(6) log D (m) = $-0.88 + 2.37$ log size (m) (*M. capensis*)

and

(7) log D (m) = $-0.91 + 2.45$ log size (m) (*M. paradoxus*).

Since hake weight is scaled to length^3, the areal density and spacing (i.e. average distance among neighbours) within two-dimensional hake shoals should be scaled approximately as the -1.5 and 0.8 power of fish weight (i.e. density $\approx f$ weight$^{-1.5}$ and spacing $\approx f$ weight$^{0.8}$).

The demonstrated size dependence of fish spacing within hake shoals is consistent with previous demonstrations of size-dependent fish spacing within shoals (e.g. Serebrov 1976; Pitcher and Partridge 1979; Rose and Leggett 1988). In addition, the $-3/2$ scaling of areal fish density to fish weight resembles that describing the self-thinning rule in plants (e.g. Gorham 1979). The scaling of shoal spacing within hake aggregates to hake length is very similar to previously published equations describing the general relationship between spacing and organismal size for a variety of terrestrial and aquatic organisms (Duarte et al. 1987).

Fisheries Implications

These results allow the formulation of expectations on the hake catch of vessels fishing within this area. Commercial vessels do not sample the area randomly; instead they use acoustic techniques to locate hake aggregations. Hence, the catch (both in numbers and biomass) of single trawls by commercial vessels should be dependent on the average size of the fish in the shoals sampled, and not on their overall abundance in the sampling area. The numbers of fish caught should be approximately scaled to the -5.0 power of fish length (Eq. 2–5), and because fish biomass is the product of fish weight (approximately length^3) and density, the biomass harvested should scale as the -2 power of fish length within the shoals.

The nonlinear scaling between hake density and biomass and fish size has other important fisheries implications. Because hake size increases with depth (Gordoa 1989; Macpherson and Duarte 1991), vessels fishing in shallow waters will sample smaller fish than vessels trawling on deeper waters. Since areal fish density changes as the -5 power of fish length, shallow trawls will generate disproportionately larger mortality (i.e. reduction in fish number) than deeper trawls. Further, since biomass, which is linearly related to fish potential reproductive capacity (cf. Duarte and Alcaraz 1989), changes as the -2 power of fish length, shallow trawls are likely to induce a dis-

proportionately greater reduction in potential recruitment than deeper trawls. Moreover, our results demonstrate that the assumption of proportionality between fishing mortality and fishing effort often assumed in fisheries management programmes is inadequate for the hake populations studied. The mortality induced by a given fishing effort will be strongly dependent on the size of the fish harvested.

The scaling of shoal density and internal structure to fish size described here is similar to those reported for other species (e.g. Pitcher and Partridge 1979; Rose and Leggett 1988). Size-dependent shoaling behaviour has been demonstrated for many pelagic species (Freon 1984; Csirke 1988; Pitcher et al. 1985, 1986). We thus believe the implications of our results to be (qualitatively) valid for other shoaling fish species.

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Growth, Parr–Smolt Transformation, and Changes in Growth Hormone of Atlantic Salmon (*Salmo salar*) Reared under Different Photoperiods

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Stefansson, S. O., B. Th. Björnsson, T. Hansen, C. Haux, G. L. Taranger, and R. L. Saunders. 1991. Growth, parr–smolt transformation, and changes in growth hormone of Atlantic salmon (*Salmo salar*) reared under different photoperiods. Can. J. Fish. Aquat. Sci. 48: 2100–2108.

Potential 1+ smolts of Atlantic salmon (*Salmo salar*) were reared under three light regimes: simulated natural photoperiod (LDN), continuous light (LD24:0), or a combination of continuous, low-intensity background light and a superimposed simulated natural photoperiod (dual photoperiod, LDD). Growth rate in freshwater was enhanced by LD24:0 and LDD, and changes associated with smolting (increased salinity tolerance, reduced condition coefficient) were advanced under LD24:0. Plasma growth hormone levels were initially high on LD24:0 and LDD whereas on LDN, plasma growth hormone levels increased gradually from February through April. Overall GH levels were negatively correlated with condition coefficient during the final stages of smolting. After 16 mo in seawater, there were no significant size differences among the groups. The incidence of sexual maturation as postsmolts was higher in the LD24:0 and LDD groups whereas the incidence of grilsing was higher in LDN. Results demonstrate the significant influence of photoperiod on growth and smolting in Atlantic salmon. An abrupt increase to continuous light in winter may be sufficient to advance important aspects of the parr–smolt transformation. Dual photoperiod may be a way to combine the increased growth rate observed under continuous light and the normal parr–smolt transformation associated with natural photoperiod.

De futurs tacons 1+ du saumon de l'Atlantique (*Salmo salar*) ont été élevés sous trois régimes d'éclairage : photopériode naturelle simulée (LDN), éclairage continuel (LD24:0) et éclairage diffus de faible intensité superposé à la photopériode naturelle (photopériode mixte, LDD). La croissance en eau douce a été stimulée par la LD24:0 et la LDD, et la LD24:0 a favorisé des changements associés au stade de tacon (tolérance accrue à la salinité, coefficient de condition réduit). L'hormone de croissance du plasma était d'abord présente en concentration élevée avec la LD24:00 et la LDD, tandis qu'avec la LDN, sa concentration augmentait graduellement de février à avril. Dans l'ensemble, les concentrations de l'hormone variaient inversement avec le coefficient de condition durant les derniers stades du tacon. Après 16 mo en eau salée, il n'y avait aucune différence de taille significative entre les groupes. La fréquence de maturation sexuelle au sade de post-tacon était plus élevée avec la LD24:0 et la LDD, tandis que la fréquence de castillons était plus élevée avec la LDN. Les résultats révèlent l'influence significative de la photopériode sur la croissance du saumon de l'Atlantique et le développement des tacons. Une augmentation abrupte de la l'éclairage continuel en hiver pourrait suffire à déclancher une partie importante de la transformation vers le stade de tacon. La photopériode mixte serait peut-être une façon de combiner l'augmentation de la croissance observée sous l'éclairage continuel et le passage au stade de tacon associé à la photopériode naturelle.

Received July 4, 1990
Accepted June 4, 1991
(JA635)

Reçu le 4 juillet 1990
Accepté le 4 juin 1991

The process of parr–smolt transformation of the Atlantic salmon (*Salmo salar*) involves metabolic and behavioural changes as well as development of hypoosmoregulatory ability which transform the dark, bottom-dwelling parr to a silvery, pelagic smolt, prepared for a life in the ocean (Dickhoff and Sullivan 1987; McCormick and Saunders 1987; McCormick et al. 1987; Thorpe 1987). Under natural photoperiod, thermal regime, and river flow, the developmental processes that constitute the parr–smolt transformation are synchronized to produce functional smolts.

When smolting salmonids are reared indoors and deprived of their natural environmental cues, problems may arise in relation to the orchestration of parr–smolt transformation (Wedemeyer et al. 1980; Hoar 1988). Photoperiods other than a simulated natural one may seriously affect the parr–smolt transformation (Saunders et al. 1985; McCormick et al. 1987; Björnsson et al. 1989; Stefansson et al. 1989). Extended daylength has a growth-promoting effect on juvenile Atlantic salmon (Saunders et al. 1985; McCormick et al. 1987; Stefansson et al. 1989); hence, continuous light is widely used for increasing the growth of juvenile Atlantic salmon in culture. Juvenile Atlantic salmon reared under continuous light conditions appear healthy, may show morphological signs of smolting, but often fail to grow well after transfer to seawater, indicating failure to complete parr–smolt transformation (Saunders et al. 1985; McCormick et al. 1987).

An increase in daylength has been found to trigger the increase in growth hormone levels observed during the final stages of parr–smolt transformation in Atlantic salmon (Björnsson et al. 1989), indicating that growth hormone may have a major role regarding several aspects of this complex process. A growth-promoting effect of mammalian as well as piscine growth hormones has been demonstrated in several teleost species, including salmonids (Pickford et al. 1959; Komourdjian et al. 1976a, 1976b; Donaldson et al. 1979; Gill et al. 1985; Seikine et al. 1985; Kawauchi et al. 1986; Skyrud et al. 1989). Another major effect is that of stimulating the hypoosmoregulatory ability. A concurrent increase of growth hormone levels, increased hypoosmoregulatory ability, and increased gill Na^+,K^+-ATPase activity occurs in smoltifying Atlantic and Pacific salmon (Björnsson et al. 1989; Boeuf et al. 1989; Young et al. 1989). A causal relationship is indicated, as growth hormone treatment partly restores the decreased gill Na^+,K^+-ATPase activity of hypophysectomized coho salmon (*Oncorhynchus kisutch*) (Björnsson et al. 1987).

This experiment was designed to investigate whether low-level continuous illumination and simulated natural photoperiod could be combined to stimulate growth and ensure a complete orchestration of parr–smolt transformation. Further, the aim of the study was to gain better understanding of the photoperiodic control of plasma growth hormone levels and to observe how changes in growth hormone levels correlate with other physiological changes during parr–smolt transformation.

Materials and Methods

Fish Stock and Rearing Conditions

Juvenile Atlantic salmon from the hatchery stock at Matre Aquaculture Station were kept under continuous light from first feeding in February 1987 until July 1987, when they were transferred to outdoor tanks and kept under seasonally changing temperature and light conditions. On November 21, 1987, three hundred potential 1+ smolts ranging from 10.4 to 14.7 cm were transferred to each of six experimental indoor tanks. The fish were reared in six 1-m^2 grey, covered fibreglass tanks with a rearing volume of approximately 450 L.

The freshwater flow was 15 L·min^{-1} in each tank. Water temperature decreased from 7°C in November to 2°C in the beginning of December. From December onwards the water was heated, raising temperatures to between 8 and 11°C. Commercial dry feed (Skretting Tess Elite, size 3.0G) was dispensed from automatic feeders to all groups during light hours of the simulated natural photoperiod group. The amount of feed was adjusted at weekly intervals to temperature and fish size according to Austreng et al. (1987).

Experimental Design

Three experimental light regimes were initiated on November 21, 1987, with two replicates for each treatment. One group was reared under a simulated natural photoperiod of 60°N (Light:Dark Natural, LDN) and a second group was reared under continuous light (Light:Dark 24:0, LD24:0). Light intensity of the light sources was 1400 lx. The third group was reared under a light regime combining a continuous, low-intensity (27 lx) background illumination and a high-intensity (1400 lx) simulated natural photoperiod. We refer to this light regime as a dual photoperiod (Light:Dark Dual, LDD).

The tanks were illuminated by fluorescent daylight tubes, with a computer program and electronic regulators to simulate the natural photoperiod, including twilight periods. Background illumination of the LDD group was from incandescent light bulbs. Characteristics of the different light sources are given in Stefansson and Hansen (1989).

Random samples of approximately 100 fish were collected for growth measurements on November 21 and December 23, 1987, and January 28, March 3, and April 26, 1988. The fish were anaesthetized (Benzocain, Sigma), measured for fork length to the nearest 0.1 cm, and weighed to the nearest 0.1 g. Specific growth rate in length (SGR) was calculated as $SGR = (e^g - 1)100\%$ where $g = (\ln(l_2) - \ln(l_1))(t_2 - t_1)^{-1}$ where l_1 and l_2 are mean lengths at times t_1 and t_2. Fulton's condition coefficient (Fulton 1902) was calculated from the formula $cc = 100wl^{-3}$ where w is the weight of each fish (grams) and l is the corresponding fork length (centimetres).

Blood samples for hormone analysis were collected between 09:00 and 10:30 on February 12, March 2, March 24, April 12, and May 5 in freshwater and on June 15, September 12, and December 12 in seawater. Twelve fish from each group were rapidly removed from the tank and quickly anaesthetized following the procedure of Stefansson et al. (1989). Blood was collected from the caudal vein using a heparinized syringe. The samples were centrifuged at 3000*g* and plasma was stored at −80°C for subsequent analysis.

Seawater exposure tests to assess hypoosmoregulatory ability were performed on February 4 (32.5‰, 24 h), March 10 (35.0‰, 24 h), and April 20 (37.5‰, 96 h) as described by Clarke and Blackburn (1977). Blood was sampled and plasma stored as described above. The fish were not fed 1 d prior to the test. The static water was continuously aerated throughout the tests. Temperature was kept the same as in the experimental tanks. The test groups were monitored throughout the test to record any mortalities.

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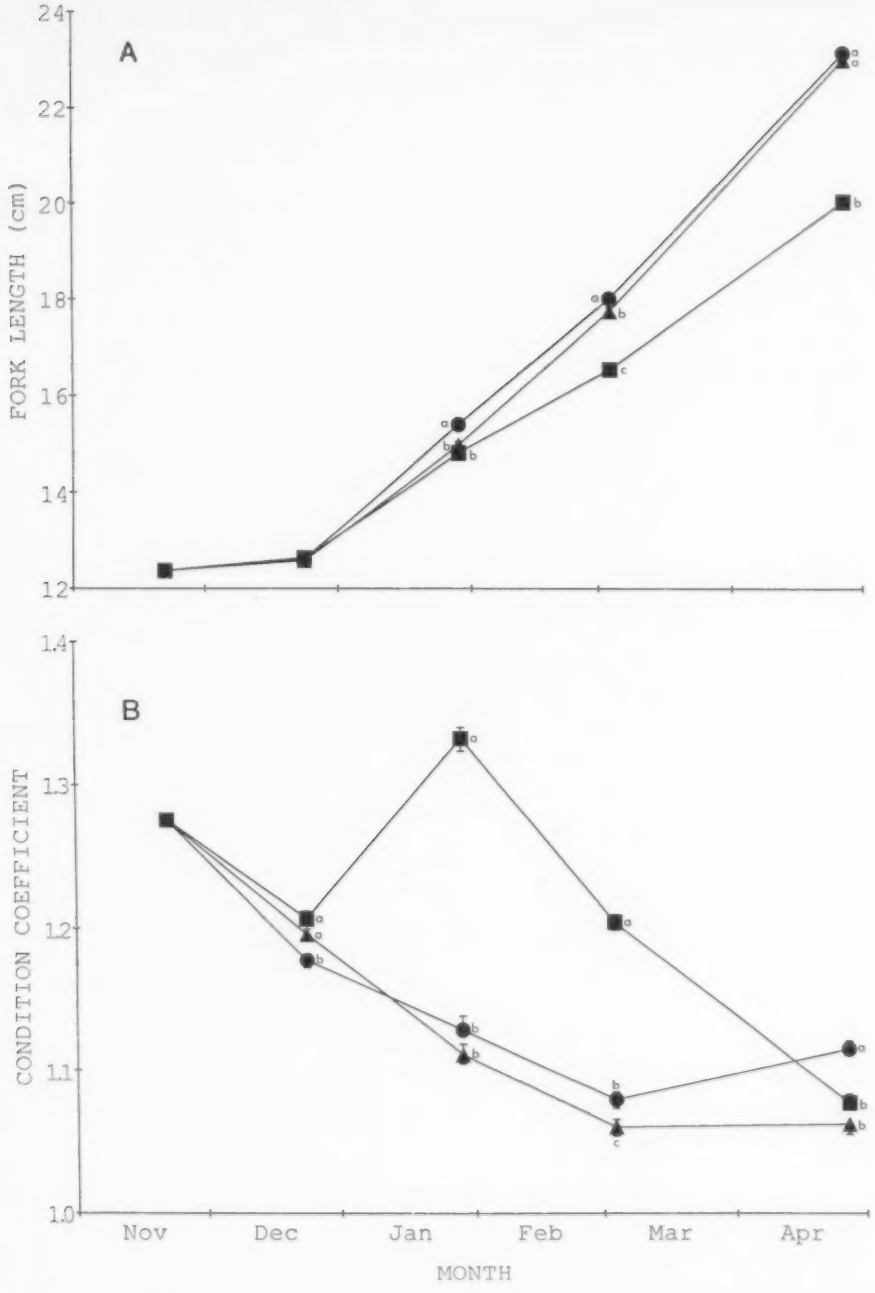

FIG. 1. (A) Fork length and (B) Fulton's condition coefficient of juvenile Atlantic salmon in freshwater under three different photoperiods (the vertical line indicating the SEM may be obscured by the symbol). Different letters indicate statistical differences ($p < 0.05$). Squares = simulated natural photoperiod (LDN), circles = continuous light (LD 24:0), and triangles = dual photoperiod (LDD).

On May 5, in preparation for transfer to seawater, the groups (replicates combined) were fin clipped (adipose or one pelvic fin) according to photoperiod treatment and the fish (n = 300–400) were transferred to a common 5-m circular tank under natural light supplied with seawater from a well. The fish were acclimated to seawater between May 5 and 12 by increasing the salinity from 10 to 20‰ after 4 d and to full-strength seawater (28–30‰) on May 12. No fish died during transfer or acclimation to seawater. Observations on survival, growth, and maturation in seawater were made on June 15, September 12,

and December 12, 1988, and March 15, June 15, and October 12, 1989. Water temperature during this period varied between 8 and 11°C.

Analytical Techniques

Plasma growth hormone levels were analyzed (2 × 50-μL samples) by a double-antibody RIA following the general protocol of Bolton et al. (1986). The primary antibody (HU-85; 7500× dilution) was raised in rabbits against recombinant chum salmon growth hormone (Kyowa Hakko Kogyo Co., Tokyo, Japan) and gives good parallel binding to Atlantic salmon plasma (B. Th. Björnsson, unpubl. results). The secondary antibody (goat anti-rabbit IgG R-0881, Sigma) was used in a 40× dilution. Native chum salmon growth hormone was used for iodination and for assay standards. Plasma chloride was analyzed (2 × 20-μL samples) using a Radiometer CMT 10 chloride titrator.

Data Analysis

Length distributions were tested for normality using a Kolmogorov–Smirnov test (Sokal and Rohlf 1981). A one-way ANOVA was used to compare mean lengths between replicates and among the experimental groups. Where differences were found among groups, Student's *t*-tests were applied. Results from the seawater challenge tests were compared using a two-tailed *t*-test. Data on maturation were tested in a chi-square test (Zar 1984). A critical level of 0.05 has been adopted unless otherwise stated.

Results

Growth Rate

As no significant differences in specific growth rate (hereafter referred to as "growth rate") were found between replicate tanks within photoperiod treatments, these data were combined in all cases.

The growth rate was low for the period early November to late December, 1987, in all groups (Fig. 1A), and increased in all groups following the increase in temperature in late December. For the period late December, 1987, to late January, 1988, the highest growth rate was observed in the LD24:0 group. For the rest of the period in freshwater the growth rate of the LDD group was equally high or higher. Although growth rate of the LDN group increased from January through April, following the increase in daylength, this group had the lowest growth rate throughout the freshwater rearing period. The mean length of the LD24:0 group was greater than that of the LDD group in January and March ($p < 0.05$) whereas in April there was no significant difference in mean length between these groups (Fig. 1A). The mean length of the LDN group was less than that of the LD24:0 and LDD groups from January onwards ($p < 0.05$).

Condition Coefficient

Following a decrease in condition coefficient in all groups from November to December, the condition coefficient of the LDN group increased to a peak in late January (Fig. 1B). The condition coefficient of the LDN group was higher than that of the LD24:0 and LDD groups in January and March ($p < 0.001$). For the LD24:0 and LDD groups the condition coefficient continued to decrease until early March. Between early March and late April the condition coefficient increased for the LD24:0 group whereas it did not change significantly in the LDD group. By the termination of the freshwater rearing period in late April, the condition coefficients of the LDN and LDD groups were not significantly different, whereas it was higher in the LD24:0 group ($p < 0.001$).

TABLE 1. Plasma chloride levels and fork lengths of juvenile Atlantic salmon following seawater challenge tests. Results are given as mean (SEM). Mean values not followed by the same letter on the same date are statistically different ($p < 0.05$).

Date	Group	Plasma chloride (mM)	Fork length (cm)
Feb. 4	LD 24:0	135.6 a (2.9)	16.1 d (0.7)
	LDN	146.4 b (3.2)	15.1 d (0.9)
	LDD	146.3 b (1.2)	15.9 d (0.4)
Mar. 10	LD 24:0	146.2 a (1.6)	18.4 d (0.3)
	LDN	139.3 b (0.5)	17.3 e (0.4)
	LDD	143.6 a (0.9)	17.9 d, e (0.3)
Apr. 20	LD 24:0	167.4 a (6.1)	22.3 d (0.4)
	LDN	141.6 b (0.5)	19.4 e (0.4)
	LDD	142.9 b (1.4)	21.7 d (0.5)

The morphological changes associated with smolting (silvering, development of black fin margins, and loose scales) occurred earlier in the LD24:0 group than in the other groups. By the termination of the freshwater rearing period (late April), all groups were classified as smolts based on their external appearance.

Plasma Chloride Levels

In early February the fish on LD24:0 had lower plasma chloride levels than the other groups following a 24-h seawater exposure test ($p < 0.05$, Table 1). The LDN and LDD groups did not differ significantly at this time. In March the LDN group had lower plasma chloride levels than the other two groups ($p < 0.05$) following a 24-h seawater exposure. In late April the LDN and LDD groups did not differ significantly following a 96-h exposure test whereas the LD24:0 group had a higher plasma chloride level ($p < 0.01$). Despite the differences in plasma chloride levels, all groups had 100% survival during the 96-h test.

Growth Hormone Levels

The levels of growth hormone were higher in the LD24:0 and LDD groups than in the LDN group during February and March (Fig. 2). From levels of approximately 15 ng·mL^{-1} in early March, the growth hormone levels of the LDD group increased to more than 40 ng·mL^{-1} 3 wk later. In mid-April, all groups showed a reduction in growth hormone levels, irrespective of photoperiod treatment. By the time of seawater transfer in early May, the mean growth hormone levels ranged from 16 to 28 ng·mL^{-1} for all groups. The growth hormone levels increased in the LDN group following seawater transfer (Fig. 2) whereas the levels were lower (<15 ng·mL^{-1}) for the other two groups throughout the seawater rearing period. By mid-December, all groups had low growth hormone levels (3–4 ng·mL^{-1}).

There was no clear relationship between levels of growth hormone and growth rate in any group or when considering the material as a whole. In late winter (February–March) the high levels of growth hormone in the LD24:0 and LDD groups were

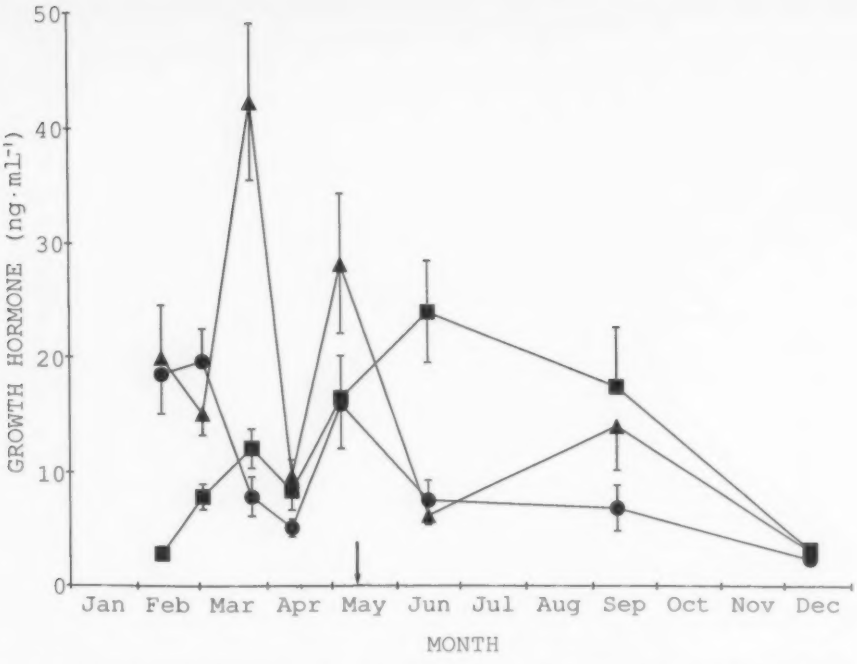

FIG. 2. Plasma growth hormone levels in juvenile Atlantic salmon in freshwater and seawater. The arrow indicates time of seawater transfer. Vertical lines indicate SEM. For definitions, see Fig. 1.

accompanied by high growth rate. Accordingly, the increase in growth rate from February through April in the LDN group was accompanied by an increase in plasma growth hormone. However, in seawater the situation was reversed, with low growth hormone levels corresponding to high growth rate. There was an overall negative correlation between condition coefficient and plasma growth hormone ($p < 0.001$, $r^2 = 0.17$) during the freshwater rearing period. The correlation was significant in the LD24:0 ($p < 0.001$, $r^2 = 0.24$) and LDN ($p < 0.001$, $r^2 = 0.19$) groups, but not in the LDD group ($p = 0.25$, $r^2 = 0.03$).

Postsmolt Performance in Seawater

The mean length increased in all groups throughout the seawater phase of the experiment (Fig. 3A). Despite their significantly smaller size at the end of the freshwater rearing period (Fig. 1A), the higher growth rate of the LDN group in seawater during late summer and fall of 1988 (Fig. 3A) allowed this group to reach a final mean length not significantly different from the others at the end of the experiment in October 1989 (Fig. 3A). The mean length of the LD24:0 group was smaller than that of the LDD group throughout the experimental period, although this was only significant in September 1988 and March 1989. At the termination of the seawater rearing period in October 1989, there were no significant differences in mean length among the groups.

The condition coefficient decreased in all groups during the first growth period in seawater (Fig. 3B). The lowest condition coefficient was seen in the LDN group and the highest in LD24:0. From June until December 1988, the condition coefficient increased in all groups, decreased again in March 1989, and increased from June until October 1989.

The percentage of fish maturing as postsmolts after 7 mo in seawater (5.7, 0.9, and 3.9% in LD24:0, LDN, and LDD, respectively) and as grilse (10.6, 16.8, and 9.1%, respectively) indicated that photoperiod treatment in freshwater may have influenced the incidence of early maturation. The LD24:0 and LDD groups, which were larger than the LDN group on transfer to seawater, had a higher incidence of males maturing as postsmolts compared with controls ($p < 0.05$). However, after 16 mo in seawater the LDN group had the highest incidence of grilse maturation (both males and females) compared with the LD24:0 and LDD ($p < 0.1$).

Discussion

Continuous light (LD24:0) enhanced growth rate in the freshwater period of this study, in accordance with previous studies on Atlantic salmon (Saunders et al. 1985, 1989; Saunders and Henderson 1988; Saunders and Harmon 1990; McCormick et al. 1987; Stefansson et al. 1989). The dual photoperiod (LDD) increased growth rate to the same extent as LD24:0. The earlier increase in growth rate of the LD24:0 group suggests that the continuous light of high intensity (1400 lx) is a stronger stimulus for growth than the continuous light of low intensity (27 lx) combined with a simulated natural photoperiod (LDD). The differences, however, were only significant from January until March.

The rapid growth on LD24:0 and LDD was accompanied by an early reduction in condition coefficient. The transient rise in condition coefficient under LDN in late January was probably in response to the increase in temperature from mid-December. Temporal changes in condition coefficient may indicate changes in important metabolic aspects of the parr–

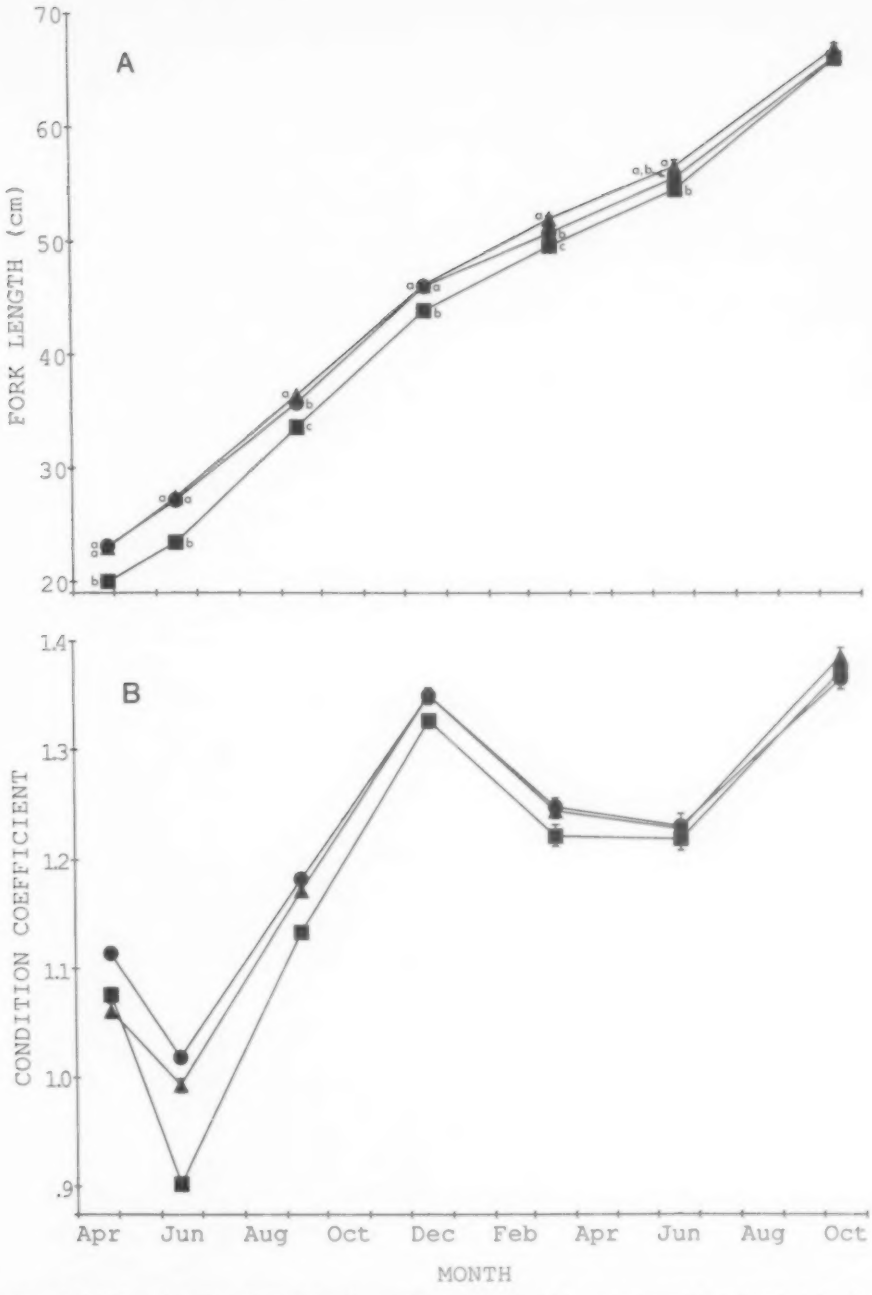

FIG. 3. (A) Fork length and (B) Fulton's condition coefficient of seawater-reared postsmolt Atlantic salmon previously exposed to three different photoperiods (the vertical line indicating the SEM may be obscured by the symbol). Different letters indicate statistical differences ($p < 0.05$). For definitions, see Fig. 1.

smolt transformation (Farmer et al. 1978), particularly the depletion of lipids in several tissues (Sheridan et al. 1983; Sheridan 1986) and the nonproportional lengthening of the caudal peduncle (Winans and Nishioka 1987). Continuous light and dual photoperiod advanced important aspects of smolting, and these groups therefore responded to the increase in temperature by a more rapid growth in length than in weight whereas the fish on LDN, which were not so advanced, did not show the same growth response in length. The subsequent increase in condition coefficient of the LD24:0 group suggests that these fish had completed certain phases of the parr–smolt transformation earlier than normal and were losing important

smolt characteristics, a situation often observed when smolts are retained in freshwater (Wagner 1974; Farmer et al. 1978; Hoar 1988). Although the temporal changes in condition coefficient were different between the LDN and LDD groups, the low condition coefficients in both groups in late spring indicated that they had completed the metabolic changes constituting an important part of the parr–smolt transformation.

The differences in plasma chloride levels following seawater exposure tests suggest a different development in hypoosmoregulatory ability among the groups. The early increase in hypoosmoregulatory ability agrees with the early reduction in condition coefficient and early development of morphological smolt characters on LD24:0 and indicates an earlier development of smolt status in this group. Similar findings have been presented by Duston and Saunders (1990) in Atlantic salmon smolts following an increase in daylength from LD8.25:15.75 to LD16:8 during late winter and spring. The early development of salinity tolerance observed in the LD24:0 group is consistent with observations of an early increase in Na^+,K^+-ATPase activity and salinity tolerance in Atlantic salmon of similar size under similar photoperiod and temperature conditions (V. A. Solbakken and S. O. Stefansson, unpubl. results). The subsequent reduction in hypoosmoregulatory ability coincides with an increase in condition coefficient, indicating that the fish were losing some of their smolt characteristics and readapting to freshwater (Folmar et al. 1982). In contrast, the groups on LDN and LDD achieved high salinity tolerance later in the spring. Despite their high growth rate throughout the freshwater rearing period, the fish on LDD developed high salinity tolerance at the same time as the controls. First-feeding fry reared under a dual photoperiod exhibited daily vertical movements similar to those observed under a simulated natural photoperiod (Stefansson et al. 1990), suggesting that the fish on LDD were responding to the cyclical changes in light intensity of the LDD regime. This behavioural pattern indicated that the fish on LDD experienced the increase in photoperiod, ensuring a proper synchronization of the parr–smolt transformation.

The early increase in growth rate, hypoosmoregulatory ability, and reduction in condition coefficient of the LD24:0 group is consistent with earlier data (Björnsson et al. 1989; Duston and Saunders 1990) supporting the hypothesis that an abrupt increase in photoperiod serves to entrain an endogenous circannual rhythm controlling important aspects of the parr–smolt transformation. All groups eventually developed important smolt characteristics (increased hypoosmoregulatory ability and reduced condition coefficient). The fish on LD24:0 achieved these aspects of smolt status earlier than the fish on LDN, in contrast with previous findings on Atlantic salmon. LD24:0 may inhibit or delay smolting when the treatment is started during first feeding (McCormick et al. 1987) or in August (Specker et al. 1989). The period of short day prior to LD24:0 (mid-November) in the present experiment may have allowed the fish to respond to the increase in photoperiod. If a period of exposure to short day photoperiod is required, the differences in photoperiodic history may explain the differences in response to LD24:0 (Clarke et al. 1989).

The lower overall growth rate of the LD24:0 group in seawater during summer and early autumn compared with the LDN group suggests that (i) the parr–smolt transformation was not completed under LD24:0, (ii) the time of seawater transfer (early May) was poorly synchronized with the peak in smolt status in the LD24:0 group, and (iii) the continuous light advanced endogenous rhythms, thereby advancing the annual growth pattern.

The rapid decrease in condition coefficient and increase in hypoosmoregulatory ability together with the high plasma growth hormone levels early in the experiment and the rapid morphological changes in the LD24:0 group support the hypothesis that an early increase in daylength advances endogenous rhythms controlling the parr–smolt transformation in Atlantic salmon. Consequently, the time of seawater transfer may have been too late to correspond with the peak in smolt status in the LD24:0 group. Also according to this hypothesis, the lower growth rate of the LD24:0 and LDD groups in seawater in autumn and winter is a consequence of an advancement of an endogenous circannual rhythm controlling growth rate. However, the high overall growth rate and negligible mortality in seawater in the present experiment is comparable with completely smoltified fish under similar conditions (Stefansson and Hansen 1989), suggesting that all groups developed important smolt characters for survival and growth in seawater.

The higher percentage of fish maturing as postsmolts in the LD24:0 and LDD groups suggests that environmental conditions in freshwater may influence the time at which the fish reach maturity. The enhancement of growth rate in freshwater, induced by continuous light, together with the large smolt size and possible alteration of endogenous rhythms in these groups has advanced the maturation process of large male smolts from LD24:0 and LDD compared with the smolts from LDN. However, the higher growth rate observed in the LDN group during the first autumn and early winter in seawater may have allowed more fish to mature as grilse the following autumn. In addition to the variation in proportion of early maturing fish among the groups, the differences in timing of early maturation (as postsmolt or grilse) may be important to the salmon farming industry. Early maturation of Atlantic salmon means lower growth rate, reduced prices, and increased mortality.

The LDD and LD24:0 groups had generally higher growth hormone levels early in the period studied whereas in the LDN group the highest growth hormone levels were seen in late spring and early summer. This correlates well with the rapid growth of the LD24:0 and LDD groups in late winter and early spring. It should be noted that a transient decrease in growth hormone levels in all groups in mid-April coincided with a period of poor water quality (low pH, high $[Al^{3+}]$). High growth hormone levels in the LDD and LD24:0 groups in February and March also coincided with a rapid decrease in condition coefficient in both groups. Similarly, increased growth hormone levels of LDN group from March onwards were concurrent with a reduction of condition coefficient and the development of hypoosmoregulatory ability in this group.

The observed overall correlations between high growth hormone levels, high growth rate, high hypoosmoregulatory ability, and decreased condition coefficient during the parr–smolt transformation agree with earlier data on smoltifying Atlantic salmon (Björnsson et al. 1989). A direct physiological relationship between growth hormone and these smoltification-related changes has been further indicated in studies of growth hormone treatment. Thus, growth hormone increases growth in several salmonid species (cf. Donaldson et al. 1979; Skyrud et al. 1989). Growth hormone reduces lipid stores and increases lipase activity in different tissues of juvenile coho salmon (Sheridan 1986). Increased catabolism is probably an important reason for decreased condition coefficient during the parr–smolt transformation (McCormick and Saunders 1987). Growth hor-

mone increases hypoosmoregulatory activity in amago salmon (*Oncorhynchus rhodurus*) (Miwa and Inui 1985) and rainbow trout (*Oncorhynchus mykiss*) (Bolton et al. 1987a). Further, treatment with growth hormone prevents the decrease in gill Na^+,K^+-ATPase activity following hypophysectomy of *O. kisutch* (Björnsson et al. 1987).

It is important to note that the above correlations are not without exceptions. The correlation between plasma growth hormone levels and condition coefficient during the final stages of smolting from February to May was significant in the LD24:0 and LDN groups only. In mid-June, after 40 d in seawater, the condition coefficient decreased in all groups whereas increased growth hormone levels were seen only in the LDN group. Conversely, growth hormone levels can increase in Atlantic salmon smolts subjected to an early increase in daylength, without a concurrent decrease in condition coefficient (Björnsson et al. 1989). Although concurrent increases in plasma growth hormone levels and growth rate have been observed in smoltifying Atlantic salmon following increased daylength (Björnsson et al. 1989), plasma growth hormone levels in salmonids often fail to correlate positively with the growth rate of the fish (Clarke et al. 1989). The high growth hormone levels of the LDD group in February and March were not accompanied by an increase in hypoosmoregulatory ability. Thus, it seems clear that although growth hormone is of importance for several physiological functions, a complex interaction between growth hormone and several other hormones is probably necessary for the successful completion of the parr–smolt transformation.

Further, an obvious breakdown of a positive correlation between growth rate and growth hormone levels is seen in the seawater postsmolts of this study, with concurrent high growth rate and low growth hormone levels. A notable converse situation is found in stunted Atlantic and coho salmon in seawater, where growth is negligible while growth hormone levels are greatly increased (Bolton et al. 1987b; Björnsson et al. 1988). This indicates that growth-promoting effects of growth hormone may be highly dependent on factors other than growth hormone, such as receptor densities, clearance rates, and interactions with other hormones, and not only on the levels of growth hormone in plasma, or that factors other than growth hormone control growth rate in the seawater phase of the life cycle.

This study has demonstrated that photoperiod treatment in freshwater may alter growth rate, timing of the parr–smolt transformation, and growth hormone levels. Our findings support the hypothesis that photoperiod treatment phase shifts seasonal cycles affecting growth and smolting. The dual photoperiod enhanced growth rate in freshwater, still allowing the fish to respond to a simulated natural photoperiod. Changes in plasma growth hormone during parr–smolt transformation influenced growth rate and condition coefficient. However, no relationship between growth hormone and growth rate was found in seawater.

Acknowledgements

The authors would like to thank the staff at Matre Aquaculture Station for skilled technical assistance. We also thank Ms. Inga Maj Örbom and Ms. Gunilla Eriksson for excellent technical assistance during the GH-RIA work. We are greatly indebted to Prof. Hiroshi Kawauchi for supplying native chum salmon growth hormone used as assay standards and iodinated label in the GH-RIA and to Dr. Akihiko Hara for supplying recombinant chum salmon growth hormone antiserum used in the GH-RIA. We thank Jim Duston for helpful suggestions and criticism during preparation of the manuscript. This study was financed by the Nordic Fund for Technology and Industrial Development (NI88.107), the Norwegian Fisheries Research Council (NFFR V.701.146), and the Swedish Natural Science Research Council (NFR B-BU 4495-105).

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Analysis of Nonlinearity Effects in Expected-Value Parameterizations of the von Bertalanffy Equation[1]

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Cerrato, R. M. 1991. Analysis of nonlinearity effects in expected-value parameterizations of the von Bertalanffy equation. Can. J. Fish. Aquat. Sci. 48: 2109–2117.

Using curvature measures, this study provides direction on exploiting the full potential of two expected-value parameterizations of the von Bertalanffy equation in reducing nonlinearity effects. This study also examines the relationship between the curvature measures and characteristics of growth data for these parameterizations. Empirical analysis of a diverse set of growth data suggests that, on average, a threefold reduction in the maximum parameter effects curvature can be achieved by simply defining one parameter as the expected mean size of an intermediate age-class, rather than as the oldest age-class in the sample. It is also found that samples in which there are proportionally more size measurements in the younger age-classes relative to the older age-classes tend to have lower nonlinearity effects. Results can be applied to existing growth data which would otherwise yield unacceptably biased and nonnormal parameter estimates and to reduce the effort required in producing accurate confidence regions.

En se basant sur des quantifications de la courbure, l'auteur explique comment exploiter le plein potentiel de deux paramétrages des valeurs probables de l'équation de von Bertalanffy pour ce qui est de la réduction des incidences du caractère curviligne. Il examine aussi la relation entre les quantifications de la courbure et les caractéristiques des données sur la croissance dans le cas de ces deux paramétrages. En moyenne, les résultats d'une analyse empirique d'une série variable de données sur la croissance portent à croire que l'on peut obtenir une réduction par un facteur de trois de la courbure maximum engendrée par les incidences des paramètres en définissant tout simplement un paramètre comme la taille moyenne probable d'une classe d'âges intermédiaire, au lieu de la plus vieille classe d'âges de l'échantillon. De plus, les échantillons comprenant proportionnellement plus de quantifications de taille des poissons des plus jeunes classes d'âges par rapport aux classes d'âges plus avancés ont tendance à présenter une plus faible incidence du caractère non linéaire. Les résultats obtenus peuvent être appliqués aux données actuelles sur la croissance qui donneraient autrement des estimations non normales des paramètres, en plus d'être biaisées de façon inacceptable. Ils peuvent aussi servir à réduire l'effort requis pour obtenir des régions de confiance précises.

Received February 26, 1990
Accepted May 9, 1991
(JA493)

Reçu le 26 février 1990
Accepté le 9 mai 1991

Despite its mathematical simplicity, use of the von Bertalanffy equation to describe growth in fish and shellfish has been criticized on a number of grounds. Firstly, it has been considered a difficult model to fit to data (Roff 1980). The availability of iterative, nonlinear regression techniques has partially resolved this problem (Gallucci and Quinn 1979; Vaughan and Kanciruk 1982). Even so, convergence to the least squares solution is not guaranteed, and good initial parameter estimates are required. Secondly, the biological interpretation of the von Bertalanffy parameters has often been questioned (Ricker 1975; Schnute and Fournier 1980; Moreau 1987). Two of the parameters lie outside the range of observations (Knight 1968; Roff 1980; Schnute and Fournier 1980), while the remaining parameter, which regulates the change in size with age, is not a true growth rate (Ricker 1975). Finally, and perhaps most seriously, concerns have been raised regarding the statistical properties of the parameter estimates (Ratkowsky 1983, 1986). Even when the von Bertalanffy equation is fit to size data which are normally distributed, the resulting parameter estimates are generally biased and nonnormal (Ratkowsky 1983, 1986). This outcome is a consequence of the nonlinearity of the model.

Bates and Watts (1980) proposed two measures, the maximum intrinsic curvature (Γ^N) and the maximum parameter effects curvature (Γ^T), to evaluate the degree of nonlinearity in a given "model – experimental design – parameterization combination." Both measures assess the adequacy of replacing the solution locus, a subspace representing all possible combinations of parameter values, with a linear, tangent plane approximation (Bates and Watts 1980). The maximum intrinsic curvature (Γ^N) measures the maximum curvature of the solution locus in a direction normal to the tangent plane. This curvature measure depends only on the model and the experimental design, and it is independent of the specific parameterization used. The maximum parameter effects curvature (Γ^T) measures the maximum degree of twisting, lack of parallelism, and unequal scaling in the parameter curves of the solution locus projected onto the tangent plane (Bates and Watts 1981). This measure is affected by the specific form of the model and may be altered by reparameterization.

When either curvature measure is large when compared with a criterion suggested by Bates and Watts (1980), the tangent plane approximation is not adequate. Consequently, it can be

[1]Contribution No. 807 of the Marine Sciences Research Center.

expected that the model – experimental design – parameterization combination will affect the convergence properties of least squares algorithms and the statistical properties of the parameter estimates (Ratkowsky 1983, 1986). If both measures are small, the tangent plane approximation to the solution locus is satisfactory, and the model – experimental design – parameterization combination exhibits what Ratkowsky (1983) has called "close-to-linear" behavior. In this case, nonlinear regression algorithms require less accurate initial values and will rapidly converge to the least squares estimate. Additionally, the resulting parameter estimates will be almost unbiased and approximately normal.

Using a variety of data sets, Ratkowsky (1983, 1986) and Cerrato (1990) have calculated curvature measures for both the common form of the von Bertalanffy equation and a number of its reparameterizations. Their results indicate that the maximum intrinsic curvature is generally low except for extremely small samples (e.g. $n = 5$). However, for all of the data sets examined, the common form of the von Bertalanffy equation was found to have unacceptably high values for the maximum parameter effects curvature. In fact, compared with all of the parameterizations considered to date, the common form of the von Bertalanffy equation has been generally the worst behaved.

Ratkowsky (1986) and Cerrato (1990) also found that reparameterizations involving what Ross (1978) has termed expected-value parameters were generally the best behaved, yielding the lowest values of Γ^T. One of these reparameterizations, first introduced by Schnute and Fournier (1980), replaces two of the parameters in the growth model with the expected mean sizes of the youngest and oldest age-classes in the sample. A second form replaces all three von Bertalanffy parameters with expected mean sizes. These reparameterizations are algebraically somewhat cumbersome, but they are promising as growth functions, since their use appears to address the concerns raised against the von Bertalanffy equation. Low curvature measures imply good convergence properties and parameter estimates which are almost unbiased and normal. In addition, the parameters themselves are expected values of observed quantities, so their biological interpretation is readily apparent.

While the curvature measures of Bates and Watts (1980) are useful in quantifying nonlinearity effects, little direction is available in choosing an experimental design or a parameterization which will produce low curvature. Analyses comparing the relative merits of different experimental designs have not been carried out for any specific nonlinear model. Analytical methods for obtaining parameterizations which yield low curvature measures were examined by Bates and Watts (1981). These methods were generally found to be impractical, either because they were difficult or impossible to solve or because the resulting parameters were not readily interpretable. In the one approach which was practical, Bates and Watts (1981) suggested choosing a transformation of restricted form and "tuning" it to minimize Γ^T for the specific experimental design being considered. They also noted that expected-value parameterizations can be used advantageously in this way.

The purpose of this study was to utilize the curvature measures of Bates and Watts (1980) to carry out a detailed evaluation of two expected-value parameterizations of the von Bertalanffy growth curve. It was undertaken to fully assess the potential of these parameterizations in reducing nonlinearity effects. It was also undertaken to clarify how specific characteristics of growth data influence the curvature measures. Throughout this study, the need to retain parameters which are interpretable in biological terms was emphasized.

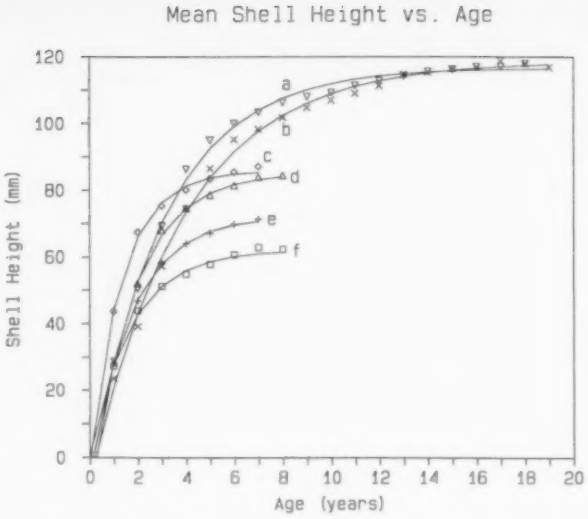

FIG. 1. Mean shell height (points) versus age for selected surf clam data sets (data from Keith 1985). Fit to the von Bertalanffy equation shown as solid lines. (a) $n = 294$, $\sigma = 7.89$, $H_\infty = 116.81$ mm, $k = 0.324$ yr^{-1}, $t_0 = 0.150$ yr; (b) $n = 135$, $\sigma = 6.57$, $H_\infty = 118.72$ mm, $k = 0.257$ yr^{-1}, $t_0 = 0.243$ yr; (c) $n = 150$, $\sigma = 3.71$, $H_\infty = 85.89$ mm, $k = 0.743$ yr^{-1}, $t_0 = 0.029$ yr; (d) $n = 43$, $\sigma = 8.96$, $H_\infty = 85.88$ mm, $k = 0.560$ yr^{-1}, $t_0 = 0.280$ yr; (e) $n = 142$, $\sigma = 6.27$, $H_\infty = 72.37$ mm, $k = 0.568$ yr^{-1}, $t_0 = 0.128$ yr; (f) $n = 128$, $\sigma = 5.27$, $H_\infty = 62.38$ mm, $k = 0.574$ yr^{-1}, $t_0 = -0.028$ yr.

Materials and Methods

Due to their very nature, any analysis evaluating curvature measures must be based on explicit sets of data. In the present study, experimental designs, parameter estimates, and error variances were taken either directly from or closely based on the characteristics of 16 samples in a surf clam (*Spisula solidissima*) study by Keith (1985). Keith measured shell height from annual increments in radially sectioned valves. All height measurements were taken at integer ages, and the variation in size with age was homoscedastic. Sample sizes ranged from 34 to 294 height versus age measurements, and the oldest individuals in each sample varied from 6 to 22 yr old. Considerable differences in surf clam growth were reported (e.g. Fig. 1), and the data, when fitted to the von Bertalanffy equation, produced a wide range of parameter estimates ($60 < H_\infty < 120$ mm, $0.25 < k < 0.75$ yr^{-1}, $-0.25 < t_0 < 0.5$ yr, $13.37 < s^2 < 80.28$). The samples in Keith's study were, therefore, ideal for a data-dependent analysis, since they spanned a broad set of characteristics. This diversity allowed the identification of general trends without having to extrapolate any characteristic and risk producing an experimentally or biologically unrealistic case.

The von Bertalanffy growth curve is given as

$$(1) \quad h = H_\infty (1 - e^{-k(t - t_0)})$$

where h is the shell height at age t, H_∞ is the asymptotic maximum size, k is a growth coefficient, and t_0 is an initial con-

dition corresponding to the age when $h = 0$. Schnute and Fournier (1980) proposed a reparameterization of the von Bertalanffy equation based on the following set of parameters (with notational differences):

$$(2)\quad h_A = H_\infty (1 - e^{-k(A - t_0)})$$

$$(3)\quad h_M = H_\infty (1 - e^{-k(M - t_0)})$$

$$(4)\quad \kappa = e^{-k}.$$

Here, h_A and h_M are expected-value parameters and represent the expected mean sizes at ages A and M, respectively ($A < M$). As defined by Schnute and Fournier (1980), A and M were taken to be the ages of the youngest (A) and oldest (M) individuals in the sample. They also assumed that successive age-classes were separated by exactly 1 yr. Substituting these relations into equation 1 yields the following reparameterization (Schnute and Fournier 1980):

$$(5)\quad h = h_A + (h_M - h_A)\frac{1 - \kappa^{t-A}}{1 - \kappa^{M-A}}.$$

A second reparameterization of the von Bertalanffy growth curve can be obtained by replacing equation 4 with a third expected-value parameter of a form similar to that in equations 2 and 3. The age chosen for this parameter is not arbitrary, however, since the overall set of transformations is generally not invertible in a closed form. Ross (1978) determined that the expected-value transformation can be inverted if the third parameter is defined at the midpoint between A and M, i.e.

$$(6)\quad h_{(A+M)/2} = H_\infty (1 - e^{-k((A+M)/2 - t_0)}).$$

Inverting equations 2, 3, and 6 and substituting yields the second reparameterization:

$$(7)\quad h = h_A + (h_M - h_A)\frac{1 - \left(\dfrac{h_M - h_{(A+M)/2}}{h_{(A+M)/2} - h_A}\right)^{2(t-A)/(M-A)}}{1 - \left(\dfrac{h_M - h_{(A+M)/2}}{h_{(A+M)/2} - h_A}\right)^{2}}$$

Schnute and Fournier (1980) defined A and M explicitly as the age of the youngest and oldest individuals in the sample. However, expected-value transformations, and the reparameterizations they define, are more general in nature and need not be limited to these choices. Restrictions in the choice of A and M fall into three separate categories. The first of these are mathematical restrictions required to make equations 5 and 7 valid reparameterizations. Here, very little is required. For equation 5, A must be finite and $A < M$. For equation 7, A and M must be finite and unequal.

The second category represents statistical considerations. It is desirable to choose A and M in a way which minimizes the maximum parameter effects curvature. Bates and Watts (1981) suggested that this may be accomplished by tuning the expected-value parameters. In the present case, this means adjusting A and M to minimize Γ^T for the particular experimental design and parameterization being considered. In turn, this process would minimize the nonlinearity effects on the statistical properties of the parameter estimates.

Within the third category are constraints which can be imposed to preserve the biological interpretation of the expected-value parameters. These are satisfied by choosing A and M to represent ages within the range of the data and also to correspond to observed age-classes. The latter constraint applied to the surf clam data implies that A and M take on integer values. Additionally, for equation 7, the sum of A and M should be even so that $(A + M)/2$ is an integer age.

The major problem addressed in the first part of this study was to identify the optimum choices for A and M which satisfy, to the extent possible, these restrictions. The method used was to first search by heuristic means for any values of A and M which minimize Γ^T for specific data sets. Next, the search was repeated but with choices of A and M limited to meet the desired biological restrictions. This procedure allowed comparisons between the overall minimum Γ^T and the smallest value consistent with the biological restrictions. In the second part of this study, characteristics of the data which affect the curvature measures were examined. These included aspects of the experimental design such as sample size, the range of age-classes present in the sample, and the age-class distribution of observations in the sample. The effect of the magnitude of the error variance and the parameter estimates were also considered. In both parts of this study, analyses were repeated for at least five different samples spanning the range of characteristics present in the surf clam data.

The computer algorithm used in this study was adapted from programs contained in Bates et al. (1983) and Ratkowsky (1983). It was tested using examples in Bates and Watts (1980) and Ratkowsky (1983). In calculating Γ^T for equation 5 with the initial conditions suggested by Bates and Watts (1980), the computer algorithm often converged on a local minimum and occasionally failed to converge at all. Calculations for this equation were repeated with various initial conditions to ensure that the global maximum was found.

Bates and Watts (1980) stated that the tangent plane closely approximates the solution locus if both the maximum intrinsic and parameter effects curvatures are "small" when compared with $1/\sqrt{F(p,v;\alpha)}$, the radius of curvature of the $(1\text{-}\alpha)$ confidence region. $F(p,v;\alpha)$ is the upper α percentile point of the F-distribution with p and v degrees of freedom. In the present study, the criterion value for assessing the magnitude of the curvature mesures was taken to be $1/2\sqrt{F(p,v;\alpha)}$. If either curvature measure exceeded this criterion value, the model – experimental design – parameterization combination was considered unsatisfactory.

Results

Tuning Analysis

The search for A and M values defining a parameterization which minimizes Γ^T in the absence of biological restrictions led to several interesting results. Values of A varied little for the surf clam samples. Choices producing a minimum in Γ^T ranged from 0.6 to 1.6 yr for equation 5 and from 1.0 to 1.6 yr for equation 7. For a given data set, the Γ^T surface was shallow in the neighborhood of the minimum. Thus, constraining A to equal 1.0 yr, i.e. the youngest age-class in the surf clam data, resulted in very modest increases in Γ^T. In fact, the effects on Γ^T were generally minor with both $A = 1.0$ yr and M constrained to meet the desired biological restrictions. For equation 5, these constraints increased Γ^T by an average of 3.5%. Increases in Γ^T averaged 20% for equation 7, varying between 8 and 36% for individual surf clam data sets. As shown below, these increases over the unconstrained minimum were quite modest. There was, therefore, little penalty associated

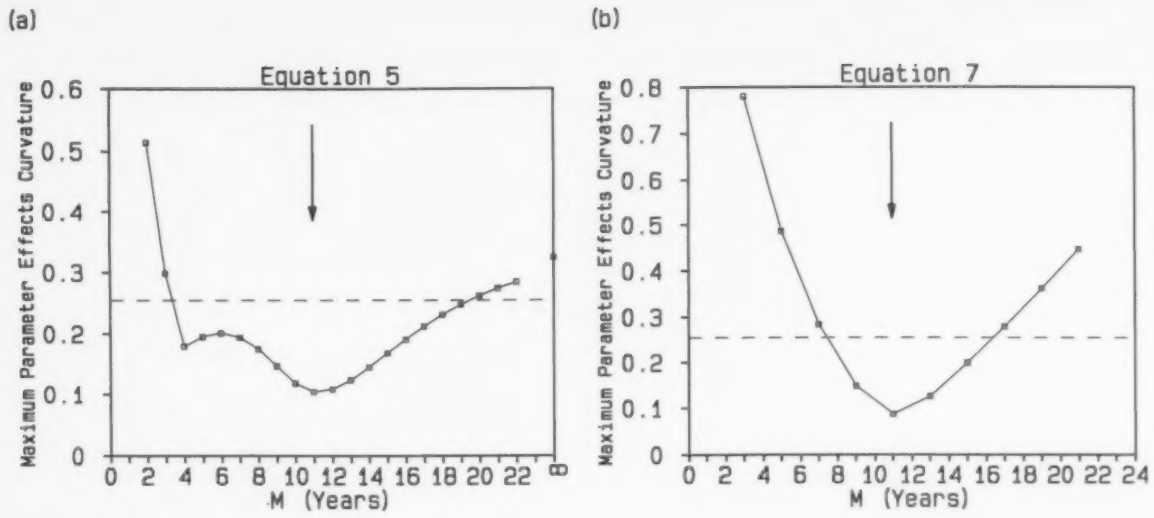

FIG. 2. Relationship between the maximum parameter effects curvature (Γ^T) and the choice of M in equation 3. (a) Expected-value parameterization in equation 5; (b) expected-value parameterization in equation 7. Results based on a surf clam data set with $n = 294$, $\sigma = 7.89$, $H_\infty = 116.81$ mm, $k = 0.324$ yr^{-1}, $t_0 = 0.150$ yr. Broken lines represent $1/2\sqrt{F}$, the criterion value for judging the magnitude of Γ^T. Arrows identify the location of the optimum parameterization.

TABLE 1. Maximum intrinsic (Γ^N) and parameter effects (Γ^T) curvatures for all 16 surf clam data sets.

		Maximum parameter effects curvature (Γ^T)			
		Equation 5		Equation 7	
Sample	(Γ^N)	Optimum	With M = oldest age-class	Optimum	With M = oldest age-class
1	0.065	0.063	0.128	0.035	0.132
2	0.116	0.110	0.320	0.071	0.192
3	0.094	0.115	0.161	0.032	0.032
4	0.069	0.074	0.162	0.033	0.148
5	0.070	0.065	0.196	0.037	0.144
6	0.096	0.103	0.201	0.054	0.185
7	0.082	0.085	0.175	0.052	0.145
8	0.051	0.042	0.117	0.055	0.135
9	0.059	0.053	0.117	0.033	0.139
10	0.055	0.052	0.142	0.044	0.224
11	0.045	0.043	0.106	0.041	0.195
12	0.143	0.132	0.446	0.084	0.289
13	0.059	0.055	0.164	0.047	0.160
14	0.205	0.179	0.420	0.220	0.251
15	0.122	0.127	0.183	0.060	0.060
16	0.103	0.081	0.464	0.047	0.233

with the desired biological restrictions, and for the remainder of the study, A was set equal to 1.0 yr and choices of M were constrained to integer values (with M odd for equation 7).

The most significant relationship observed was the variation in the maximum parameter effects curvature with respect to the choice of M. This relationship for equation 5 is illustrated by an example in Fig. 2a. Two local minima were generally present, with the second always yielding the smallest value of Γ^T. Except for an extreme case to be presented later, the optimum always occurred for a parameterization based on an intermediate age-class. For the example in Fig. 2a, the optimum was obtained by defining the expected-value parameter as $h_{11} = 113.31$ mm. A parameterization based on the oldest age-class present in the sample (e.g. $h_{22} = 116.68$ mm in Fig. 2a), as originally suggested by Schnute and Fournier (1980), generally yielded a Γ^T value which was larger than almost every other choice and which was several times greater than the optimum (Table 1). Γ^T increased for choices of M extrapolated beyond the range of the data. This increase was gradual, and even when $M \to \infty$ (i.e. $h_M = H_\infty$), the parameterization did not result in substantially greater values than obtained for the oldest age-class in the sample (Fig. 2a). Note that in the

example (Fig. 2a), defining a parameterization based on h_{11} satisfies the $1/2\sqrt{F}$ criterion, while the parameterization based on the oldest age-class (h_{22}) would be judged unsatisfactory.

The results for equation 7 were similar (Fig. 2b). However, only a single minimum in Γ^T was observed. In comparing equations 5 and 7, the location of the optimum always corresponded closely and rarely differed by more than 1 yr. The parameters in equation 7 were somewhat better behaved than those in equation 5 for the surf clam data, yielding the lowest maximum parameter effects curvature at the optimum in 14 of the 16 data sets (Table 1).

Relationship between the Curvature Measures and Characteristics of the Data

Experimental design considerations

Both the maximum intrinsic curvature and the maximum parameter effects curvature depend on a number of properties related to the experimental design. These include sample size, the range of ages in the data, and the age-class distribution of observations in the sample. The dependence of the curvature measures on sample size is simple (Bates and Watts 1980). For example, doubling the number of observations in each age-class, which does not alter any other aspect of the experimental design, reduces the curvature measures by a factor of $1/\sqrt{2}$. The value of M which minimizes Γ^T is not affected.

The range of ages in the data set affects the curvature measures in a more complex way. An example illustrating the relationship is presented in Fig. 3. To constuct this example, the number of age-classes in the sample was successively increased while the sample size was held constant. Each point in the figure represents the results from a sample consisting of c integer age-classes $(1,2,\ldots,c)$. To the extent possible, an equal number of observations was assigned to each age-class. This could only be done approximately, since dividing the sample size by the number of age-classes rarely resulted in an integer value. To account for the remainder from integer division, an additional observation was allocated to the first several age-classes.

The maximum intrinsic curvature of the model monotonically increased as the range of age-classes present in the sample was increased (Fig. 3a or 3b). The M value corresponding to the optimum maximum parameter effects curvature for the sample gradually shifted, also increasing along with the range of age-classes (Fig. 3a or 3b). The curve representing the optimum Γ^T generally had an irregular appearance. This was in large part due to limitations imposed on A and M by the desired biological restrictions. For equation 5, the optimum Γ^T declined steeply at first, but then gradually increased with the range of age-classes (Fig. 3a). For equation 7, the optimum Γ^T tended to increase but in an irregular fashion (Fig. 3b).

It should be noted that in samples consisting of a very severely restricted range of age-classes (3 yr for the oldest age-class in Fig. 3a and 5 yr for Fig. 3b), the M value corresponding to the optimum Γ^T can equal the oldest age-class present. These extreme cases are the one exception to the general relationships reported in the last section.

Of all the aspects related to the experimental design, the relationship between the curvature measures and the age-class distribution of the sample proved to be the most interesting. In fitting the von Bertalanffy growth curve, one common experimental design is to use an equal number of size measurements in each age-class. A second common situation is where the number of size measurements is proportional to relative abundance. This generally implies that the number of observations declines with age.

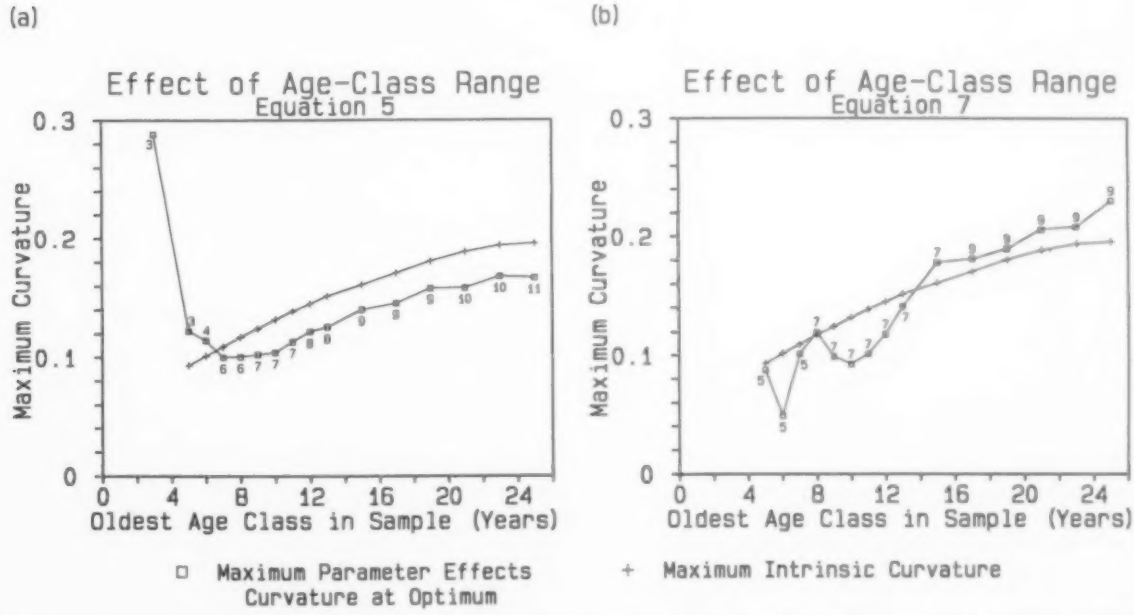

FIG. 3. Dependence of the curvature measures on the range of ages in the data set. Sample size is 128 for all cases. (a) Expected-value parameterization in equation 5; (b) expected-value parameterization in equation 7. Results based on a surf clam data set with $\sigma = 5.27$, $H_\infty = 62.38$ mm, $k = 0.574$ yr^{-1}, $t_0 = -0.028$ yr. Numbers associated with the points represent the values of M corresponding to the optimum.

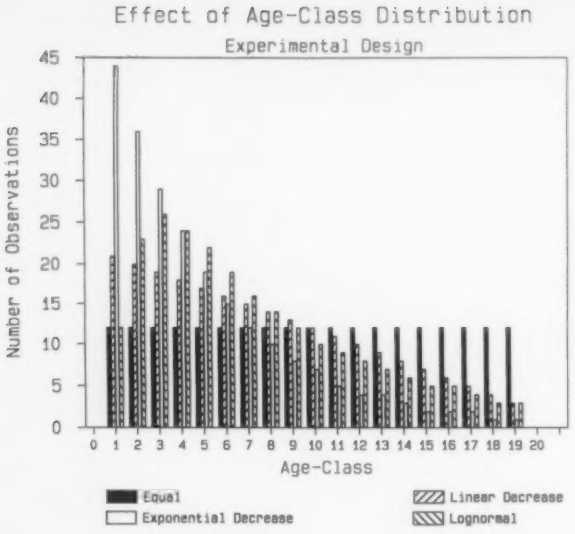

FIG. 4. Experimental designs used for examining the relationship between the distribution of observations in the sample and the maximum parameter effects curvature (see results in Fig. 5).

Several variations on these basic experimental designs were examined. The results are illustrated by example in Fig. 4 and 5. In this example, four experimental designs were constructed (Fig. 4): an equal number of observations in each age-class, a lognormal distribution, a distribution which linearly declined with age, and an exponentially decreasing distribution of observations with age. The maximum intrinsic curvature was generally greatest for the equal distribution and smallest for the exponentially decreasing distribution. In the example, Γ^N = 0.055, 0.050, 0.047, and 0.043 for the equal, lognormal, linearly decreasing, and exponentially decreasing cases, respectively.

The behavior of the maximum parameter effects curvature for equation 5 is shown in Fig. 5a. Both the magnitude and the location of the optimum were influenced by the experimental design. Like the maximum intrinsic curvature, the optimum Γ^T was generally greatest for the equal distribution and smallest for the exponentially decreasing experimental design. The behavior for equation 7 was similar (Fig. 5b).

These results suggest that samples with a larger fraction of the size measurements in the younger age-classes produce lower curvature measures. With regard to nonlinearity effects, therefore, it appears to be less advantageous to have experimental designs with an equal number of observations allocated to age-classes. This was a quite unexpected outcome because of the usual emphasis on balanced designs in statistical studies.

Dependent variable effects

Characteristics of the dependent variable as reflected in the error variance and the parameter estimates affect both the maximum intrinsic curvature and the maximum parameter effects curvature. While not under the control of the investigator, it is interesting to relate the curvature measures to these characteristics. Like sample size, the relationship between the error variance and the curvature measures is simple. Both the maximum intrinsic curvature and the maximum parameter effects curvature are directly proportional to σ. The value of M which minimizes Γ^T is not altered by changing the error variance.

To examine the relationship between the curvature measures and other characteristics of the dependent variable, an experimental design and a specific set of parameter estimates from the surf clam data were chosen. Each of the original von Bertalanffy parameters was then varied to span the range of all the surf clam samples in the study ($60 < H_\infty < 120$ mm, $0.25 < k < 0.75$ yr^{-1}, $-0.25 < t_0 < 0.5$ yr).

Both curvature measures were inversely related to t_0 and H_∞. That is, characteristics of the size data which increase either t_0 or H_∞ resulted in decreases in Γ^N and Γ^T. However, the location of the optimum Γ^T was not altered by changes in t_0 or H_∞ over the range examined. An example illustrating the effect of changing H_∞ is shown in Fig. 6 and summarized in Table 2.

Both the maximum intrinsic curvature and the optimum maximum parameter effects curvature were directly related to the growth coefficient k. Increases in k resulted in increased values of Γ^N and the optimum Γ^T for the sample. Additionally, the M value corresponding to the optimum maximum parameter effects curvature decreased with k. An example is presented in Fig. 7 and summarized in Table 2.

Discussion

As stated in the introduction, Ratkowsky (1986) and Cerrato (1990) have demonstrated that of the various reparameterizations of the von Bertalanffy growth curve examined to date, forms involving expected-value parameters have generally yielded the lowest maximum parameter effects curvature. This is a desirable property, and despite their apparent complexity in form, there are considerable benefits associated with the application of expected-value parameters. In the present study, two expected-value parameterizations (equations 5 and 7) were examined in detail. Equation 7 yielded the lowest values of Γ^T when applied to most of the original surf clam samples (Table 1). However, entirely plausible examples, whose results consistently favored equation 5, were easily constructed (see Table 2). There is, therefore, no evidence at the present time to unequivocally favor one of these two parameterizations over the other.

Throughout this study, the flexible nature of expected-value transformations has been emphasized. Equations 5 and 7 each represent a family of models. An individual parameterization within a family is defined only when a specific choice of A and M is made. In previous studies (Schnute and Fournier 1980; Ratkowsky 1986; Cerrato 1990), h_A and h_M were constrained to represent the expected mean sizes of the youngest and oldest individuals in the sample. The results of the present study indicate that this choice fails to exploit the full potential of expected-value transformations in reducing the maximum parameter effects curvature. Current results also suggest that this potential can be exploited without sacrificing the biological interpretability of the parameter estimates. There was little penalty associated with restricting A to correspond to the youngest age-class in the sample and constraining M in such a way that the remaining expected-value parameters represented observed quantities. In fact, these constraints simplify the tuning analysis considerably. The problem is reduced to trying out a small number of choices for M (e.g. the points in Fig. 2) until a minimum is found. Thus, the tuning analysis does not require specialized software beyond a program which can calculate the curvature measures.

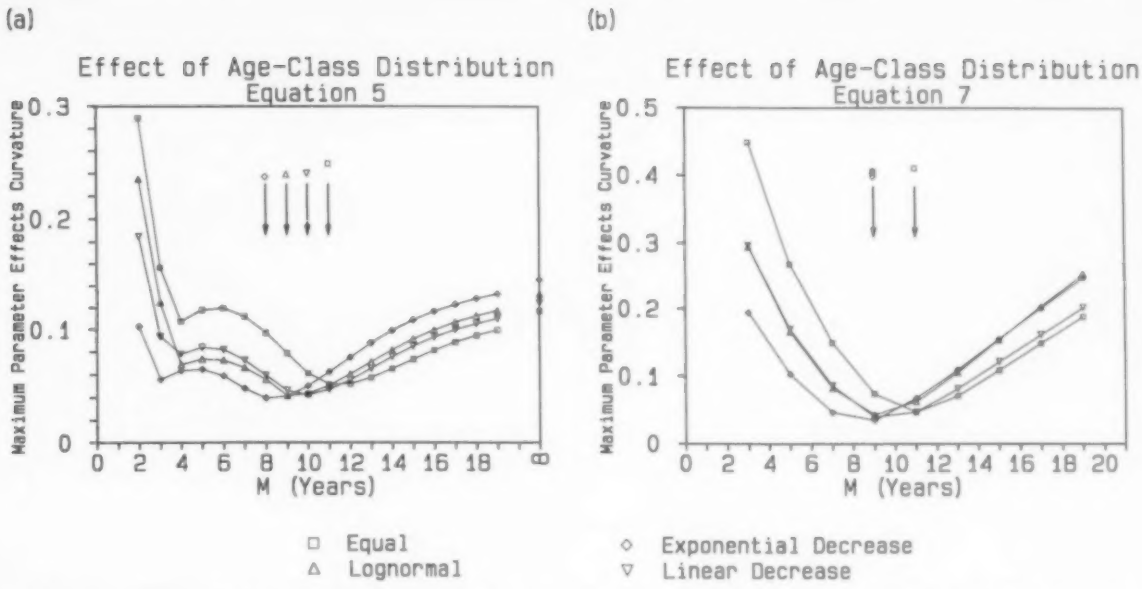

FIG. 5. Relationship between the distribution of observations in the sample and the maximum parameter effects curvature. (a) Expected-value parameterization in equation 5; (b) expected-value parameterization in equation 7. Results based on a surf clam data set with $n = 228$, $\sigma = 5.94$, $H_\infty = 114.77$ mm, $k = 0.384$ yr^{-1}, $t_0 = 0.318$ yr. Arrows with associated symbols identify the location of the optimum parameterization.

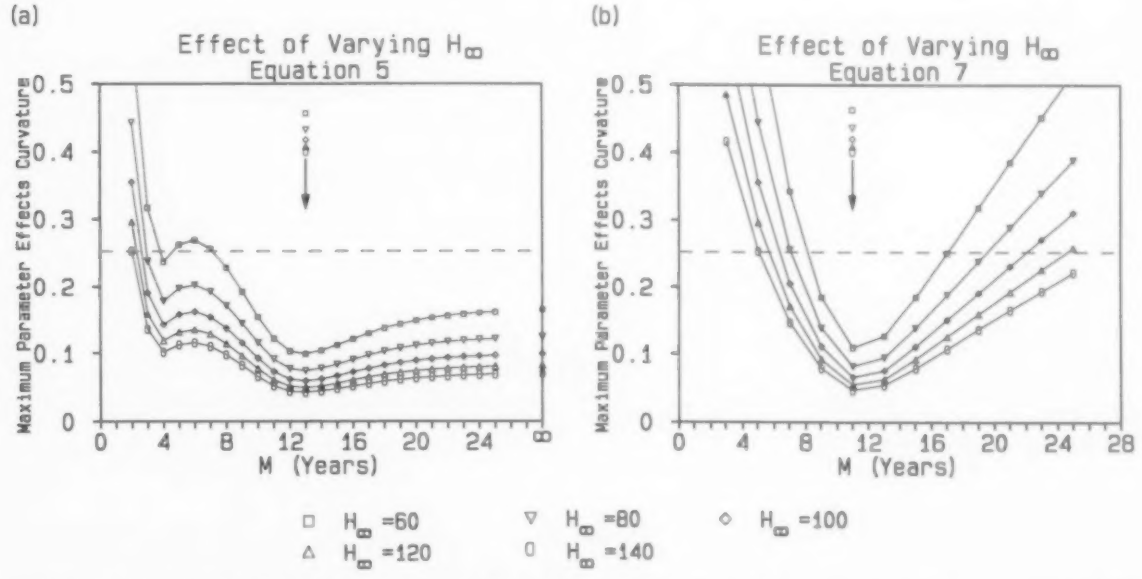

FIG. 6. Effect of varying H_∞ on the maximum parameter effects curvature. (a) Expected-value parameterization in equation 5; (b) expected-value parameterization in equation 7. Results based on a surf clam data set with $n = 150$, $\sigma = 3.71$, $k = 0.743$ yr^{-1}, $t_0 = 0.029$ yr. Broken lines represent $1/2\sqrt{F}$, the criterion value for judging the magnitude of Γ^T. Arrows with associated symbols identify the location of the optimum parameterization.

The most significant outcome of this study is the result that, with regard to statistical considerations, basing an expected-value parameterization on the oldest age-class present in the sample is a poor choice. Both equations 5 and 7 are characterized by a maximum parameter effects curvature which varies with the choice of M and which has a distinct optimum associated with an intermediate age-class. Defining h_M as the expected mean size of the oldest age-class resulted in a maximum parameter effects curvature which was greater than almost any other choice. On average, Γ^T was about three times larger than the optimum (Table 1). Additionally, for equation 5, there was little difference in the magnitude of Γ^T between a param-

TABLE 2. Effect of varying H_∞ and k on the maximum intrinsic (Γ^N) and parameter effects (Γ^T) curvatures (see Fig. 6 and 7 for further information).

Effect of H_∞		Equation 5		Equation 7	
H_∞ (mm)	Γ^N	Optimum M (yr)	Optimum Γ^T	Optimum M (yr)	Optimum Γ^T
60	0.105	13	0.099	11	0.110
80	0.078	13	0.074	11	0.082
100	0.063	13	0.060	11	0.066
120	0.052	13	0.050	11	0.055
140	0.045	13	0.043	11	0.047
Effect of k		**Equation 5**		**Equation 7**	
k (yr^{-1})	Γ^N	Optimum M (yr)	Optimum Γ^T	Optimum M (yr)	Optimum Γ^T
0.250	0.054	16	0.054	15	0.048
0.375	0.070	13	0.067	13	0.076
0.500	0.085	11	0.078	9	0.105
0.625	0.101	10	0.086	9	0.124
0.750	0.118	9	0.095	7	0.153

eterization based on the oldest age-class and one based on the asymptotic maximum size (i.e. $h_M = H_\infty$).

Taking advantage of the flexible nature of equations 5 and 7, it was always possible to reduce Γ^T to values which were comparable with and at times lower than the maximum intrinsic curvature (e.g. see Tables 1 and 2). This is an interesting result, since investigators working with the von Bertalanffy equation (Ratkowsky 1983, 1986; Cerrato 1990) and with other nonlinear models (Bates and Watts 1980; Ratkowsky 1983) have noted that the maximum intrinsic curvature of a model rarely exceeds the $1/2\sqrt{F}$ criterion. Thus, judicious use of expected-value parameters should be successful in reducing the maximum parameter effects curvature to acceptable levels in most cases.

The magnitude of the curvature measures depends on both the specific details of the experimental design and on the characteristics of the dependent variable. Curiously, the location of the optimum in Γ^T for a sample was found to be sensitive to only a few elements: the age range of the data, the age-class distribution of the sample, and the growth coefficient k. The implications of this are not fully understood at present. Conditions which elevate the magnitude of the curvature measures include small sample size, a large data range, a large error variance, small H_∞, and a high value of k. By taking these conditions in combination at their extremes, it is possible to construct examples in which both the maximum intrinsic curvature and the optimal maximum parameter effects curvature exceed the $1/2\sqrt{F}$ criterion. While technically possible, all indications from examining actual growth data, however, suggest that such cases would not arise often. If need be, empirical, predictive relationships could be developed relating the magnitude of the curvature measures to aspects of the data. The factor limiting a general chracterization of these relationships is the great variety of sample age-class distributions which are possible. If a specific application permits it, standardizing the form of the sample age-class distribution could circumvent this limitation.

Before closing, several practical aspects of the current results should be mentioned briefly. The present study indicates that the sample size requirements needed to meet the $1/2\sqrt{F}$ criterion can be decreased if the flexible nature of expected-value parameterizations is fully exploited. For example, one of the smallest samples considered, sample 12 in Table 1, consists of only 48 size measurements and $1/2\sqrt{F} = 0.243$. Γ^T for both equations 5 and 7, when parameterized with M equaling the oldest age-class in the sample, exceeds this criterion. However,

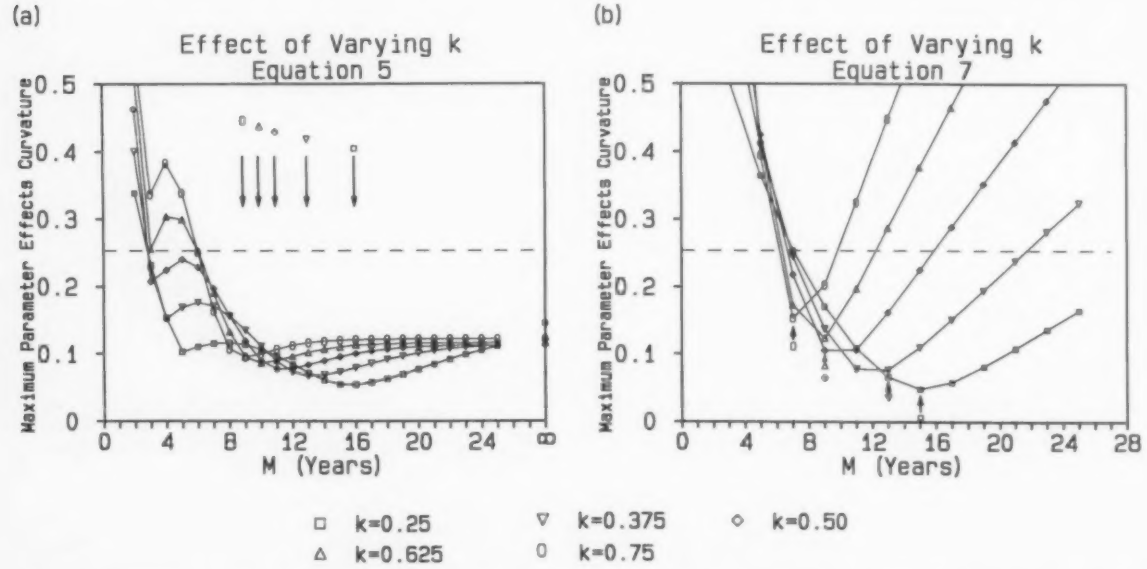

FIG. 7. Effect of varying k on the maximum parameter effects curvature. (a) Expected-value parameterization in equation 5; (b) expected-value parameterization in equation 7. Results based on a surf clam data set with $n = 150$, $\sigma = 3.71$, $H_\infty = 85.89$ mm, $t_0 = -0.029$ yr. Broken lines represent $1/2\sqrt{F}$, the criterion value for judging the magnitude of Γ^T. Arrows with associated symbols identify the location of the optimum parameterization.

the optimum parameterization yields $\Gamma^T = 0.132$ and 0.084, respectively, and the criterion for each is easily met. The present study also suggests that experimental designs with unequal allocations of observations to age-classes are effective in reducing nonlinearity effects. With respect to minimizing nonlinearity effects, there appears to be no compelling reason to require an equal number of size measurements per age-class. Experimental designs proportional to abundance, which are easier to obtain from a practical standpoint, are sufficient. It should be noted, however, that the experimental design also affects the size of the confidence region for the parameter estimates, and this effect was not assessed in the present study. The reader is cautioned, therefore, not to base decisions concerning experimental designs solely on nonlinearity criteria.

Additionally, the results of this study can be applied to the problem of generating accurate confidence regions for parameters in nonlinear models. Likelihood regions are more accurate than linear confidence regions because they are insensitive to the parameter effects curvature (Ratkowsky 1983), and based on experience, the effect of the intrinsic curvature of the model is generally small (Cerrato 1990). However, it requires a substantial computational effort to produce likelihood regions. Given the level of reduction in the maximum parameter effects curvature observed in this study, linear confidence regions generated for an optimal parameterization should be almost indistinguishable from likelihood regions. These accurate, linear confidence regions can then be transformed into another parameter set if suitable for an application and do not limit an investigator's choice of parameters. This route, while indirect, can reduce the effort involved in generating confidence regions.

This study did not consider the effects of other data characteristics such as inhomogeneous variances, the presence of dominant age-classes, or missing age-classes in the sample. These add another level of complexity to an already complicated problem. I venture to guess, however, that such characteristics will make exploiting the full potential of expected-value parameterizations even more meaningful.

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Variations in Diet, Daily Ration, and Feeding Periodicity of Pacific Hake (*Merluccius productus*) and Spiny Dogfish (*Squalus acanthias*) off the Lower West Coast of Vancouver Island

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Tanasichuk, R. W., D. M. Ware, W. Shaw, and G. A. McFarlane. 1991. Variations in diet, daily ration, and feeding periodicity of Pacific hake (*Merluccius productus*) and spiny dogfish (*Squalus acanthias*) off the lower west coast of Vancouver Island. Can. J. Fish. Aquat. Sci. 48: 2118–2128.

We examined aspects of summer feeding in Pacific hake (*Merluccius productus*) and spiny dogfish (*Squalus acanthias*). Euphausiids (*Thysanoessa spinifera, Euphausia pacifica*) and Pacific herring (*Clupea harengus pallasi*) accounted for almost all of the hake diet over all August sampling periods (1983, 1985–89) and the 1988 summer feeding period (June–October). Summer ration estimates were higher for hake feeding on the continental shelf than for fish feeding along the shelf-break. Daily ration declined over summer because the euphausiid ration dropped, even though the herring ration increased. Euphausiids, herring, and hake were the major dogfish prey items. The dogfish ration in August was 1.6 times that of hake. Both predators fed sporadically between dawn and dusk. They evacuated prey linearly over time and digested euphausiids most rapidly. Euphausiids were the most important prey. The size of herring taken varied over time in both predators. Hake took all age-classes of herring whereas dogfish tended not to feed on young-of-the-year (YOY) herring. Herring and predator size relationships were sometimes insignificant, reflecting variation in YOY herring availability. Herring became progressively more important prey as predator size increased.

Nous avons examiné des éléments de l'alimentation estivale du merlu du Pacifique (*Merluccius productus*) et de l'aiguillat commun (*Squalus acanthias*). Les euphausiidés (*Thysanoessa spinifera, Euphausia pacifica*) et le hareng du pacifique (*Clupea harengus pallasi*) comptent pour presque toute l'alimentation du merlu durant tous les échantillonnage d'août (1983, 1985–89) et la période d'alimentation estivale de 1988 (juin à octobre). Les rations estivales seraient plus élevées pour le merlu qui fréquente le plateau continental que pour celui qui s'alimente au bord du talus. La ration quotidienne diminue durant l'été parce que la baisse d'euphausiidés ne compense pas l'augmentation du hareng. Les euphausiidés, le hareng et le merlu sont les principales proies de l'aquillat. En août, sa ration est 1,6 fois celle du merlu. Les deux prédateurs se nourrissent sporadiquement entre l'aube et le crépuscule. Ils évacuent leurs proies de façon linéaire dans le temps et digèrent les euphausiidés, proie la plus importante, plus rapidement. La taille des harengs capturés varie dans le temps pour les deux prédateurs. Les merlus prennent des harengs de tous les âges tandis que l'aquillat ne semble pas se nourrir des jeunes de l'année. Les rapports de taille entre les harengs et les prédateurs étaient parfois sans signification, fluctuant avec la disponibilité des jeunes harengs de l'année. Le hareng devient une proie plus importante à mesure que la taille du prédateur augmente.

Received October 9, 1990
Accepted June 4, 1991
(JA747)

Reçu le 9 octobre 1990
Accepté le 4 juin 1991

La Perouse Bank, located off the lower west coast of Vancouver Island, is an extremely productive fishing area on the North American west coast. Commercial fish catches in the relatively small (10 000 km^2) Bank region have averaged 5.3 tonnes (t)·km^{-2}·yr^{-1} over the last 30 yr. This is about five times the average yield per unit area along the eastern boundary of the Gulf of Alaska (Ware and McFarlane 1989). Pacific hake (*Merluccius productus*) catches have averaged 52 000 t annually over the last 5 yr. Catches of sablefish (*Anoplopoma fimbria*) and other groundfish species averaged 4000–5000 t·yr^{-1} between 1984 and 1989.

Hake, spiny dogfish (*Squalus acanthias*), and Pacific herring (*Clupea harengus pallasi*) dominate the pelagic fish biomass in the La Perouse Bank region. Francis (1983) described Pacific hake as being, commercially and ecologically, a prominent fish species in the Coastal Upwelling Domain (CUD). He felt that its strongest influence in the CUD, which extends from Baja California to Vancouver Island (Ware and McFarlane 1989), might be as a predator and competitor during feeding migrations. Bailey et al. (1982) reported that about 1.5 million t of hake exist within the CUD, based on triennial stock assessments. They also described the annual northward migration to summer feeding areas. These movements are size specific. Larger fish migrate progressively further north. The largest fish occur off the west coast of British Columbia, especially in the La Perouse Bank area. Beamish and McFarlane (1985) stated that about 350 000 t of hake occurred in the Bank area in 1977 and 1980. Ketchen (1986) summarized information on the biology and commercial utilization of dogfish in the Northeast Pacific. The center of abundance appears to be the British Columbia – Washington region (48–54°N). The lower west coast of Vancouver Island is one of three areas of heavy concentration which have been commercially important. Dogfish biomass averages 50 000 t·yr^{-1} of marketable sized (>72 cm)

fish (M. Saunders, Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, B.C., pers. comm.). Results of various research surveys (e.g. Taylor 1969; Shaw et al. 1989) indicated that herring occupy the Bank area over summer. Tester (1948) suggested, based on tag returns, that herring feeding in the Bank area are predominantly fish which spawn in Barkley Sound but also include fish which spawn further up the west coast of Vancouver Island or in the Strait of Georgia. Haegele (1986) reported recent results supporting this. Herring stock assessments indicate a biomass of between 30 000 and 50 000 t off the lower west coast of Vancouver Island (Haist and Schweigert 1990). In addition, about 15 000 t of coho salmon (*Oncorhynchus kisutch*) and 11 000 t of chinook salmon (*O. tshawytscha*) feed in the La Perouse Bank area during the summer.

The La Perouse Bank Project began in 1985. It was designed to examine the physical, biological, and fisheries oceanography of the lower west coast of Vancouver Island. The goals were to get first-order estimates of the impact of interannual oceanic fluctuations on the planktonic food web, fish species, and interactions among plankton and fish. The overall objective is to understand how oceanographic and biological factors generate fish abundance trends. Special emphasis is placed on determining the impact of oceanic conditions and offshore predators on Pacific herring year-class strength. Herring is currently the most valuable fish species on the British Columbia coast because of the lucrative roe fishery.

The aim of this study was to determine the significant feeding characteristics of hake and dogfish around La Perouse Bank, with special reference to herring as prey. This paper describes inter- and intraannual variations in diet composition, daily ration, and feeding periodicity. It also provides the data needed to estimate the impact of hake and dogfish predation on herring. The assessment of this impact on herring recruitment and stock biomass will be the subject of a future paper.

Materials and Methods

Data Sources

We used data from fish collected during commercial and research trawling operations. Commercial fisheries for Pacific hake usually occur between July and October and consist of joint venture and foreign midwater trawling operations. Joint venture fisheries involve Canadian trawlers supplying foreign processing vessels with catch taken from large concentrations of hake during daylight. These aggregations usually occur on the continental shelf. Foreign vessels fish at night and further seaward, along and off the shelf-break.

Research surveys occurred in August–September 1983 and, as part of the La Perouse Project, every August since 1985. Fish were collected during daylight with commercial midwater and bottom trawl gear. Our strategy was to sample major fish concentrations in each fishing subarea (Fig. 1). We defined these subareas using bathymetric and physical oceanographic considerations and information on historic fish distributions.

Fish Sampling

Samplers recorded fork length, sex, and state of maturity (Weir et. al 1978) for each hake and total length and sex for each dogfish. All fish were examined fresh and at sea. We sampled fish collected during research cruises. Observers contracted by the Canadian Department of Fisheries and Oceans sampled fish on the foreign fishing vessels. Observers underwent extensive training in biological sampling and stomach content analysis before leaving for the fishing grounds.

Stomach content descriptions for all fish sampled consisted of identifying each prey item to broad taxonomic group, visually estimating the volume (cubic centimetres) of each item and then assigning a digestion code. Plastic syringes were used as guides for estimating prey volume. Digestion codes ranged from 1 (fresh) through 5 (fully digested). Standard lengths (millimetres) of incompletely digested herring were measured during all research cruises and by observers in 1987. Standard lengths were also estimated from intact vertebral columns of completely digested herring. Observers did not measure volume of herring in hake in 1987 or herring lengths in 1988. We used length frequency data of aged samples to group ingested herring by age-class (0+, <12 cm standard length; 1+, between 12 and 16 cm; 2+, between 16 and 18 cm; 3+ and older, >18 cm).

Estimating Daily Ration

Our daily ration estimates were calculated from mean stomach fullness indices (MSFIH for herring and MSFIE for euphausiids) for each tow, after excluding fish with everted stomachs. We converted prey volumes to weights (weight (g) = 1.08·prey volume), based on replicate volumetric measurements of weighed samples of euphausiids. (The euphausiid samples had been preserved in 5% formalin and were damp-dried before weighing.) Stomach contents were then expressed as a percentage of the body weight, or in other words, a stomach fullness index (SFI). We estimated hake body weight as $W = aL^b$, where W = total weight (grams) and L = fork length (centimetres), using weight–length parameters obtained from the research surveys:

	Parameters	
Year	a	b
1983	0.0082	2.935
1985	0.0082	2.935
1986	0.0278	2.634
1987	0.0153	2.788
1988	0.0125	2.833
1989	0.0898	2.359

The parameters for 1983 and 1985 were used to calculate body weight for fish sampled from commercial catches. There was no significant effect ($t < 0.1$, $p > 0.9$) of using the 1983–85 or year-specific parameter estimates on SFI for herring or euphausiids. Power analysis indicated that most (92.3%) of the two distributions of SFI values calculated from each length–weight parameter estimate overlapped. Dogfish SFI's were estimated using $W = 0.000127L^{3.783}$ (Saunders et al. 1984).

We estimated daily rations for each year, month, and location combination (shelf (subareas < 12), shelf-break (subareas > 11)) from smoothed values of MSFI's for herring and euphausiids in hake and for herring, euphausiids, and hake in dogfish. MSFI values were smoothed using the LOWESS algorithm. Wilkinson (1988) recommended it for smoothing ordered pair data when the X value (in our case time of day) is unequally spaced over the time series. We used one half of the points in each data set to compute each smoothed value; these points surrounded the value being smoothed.

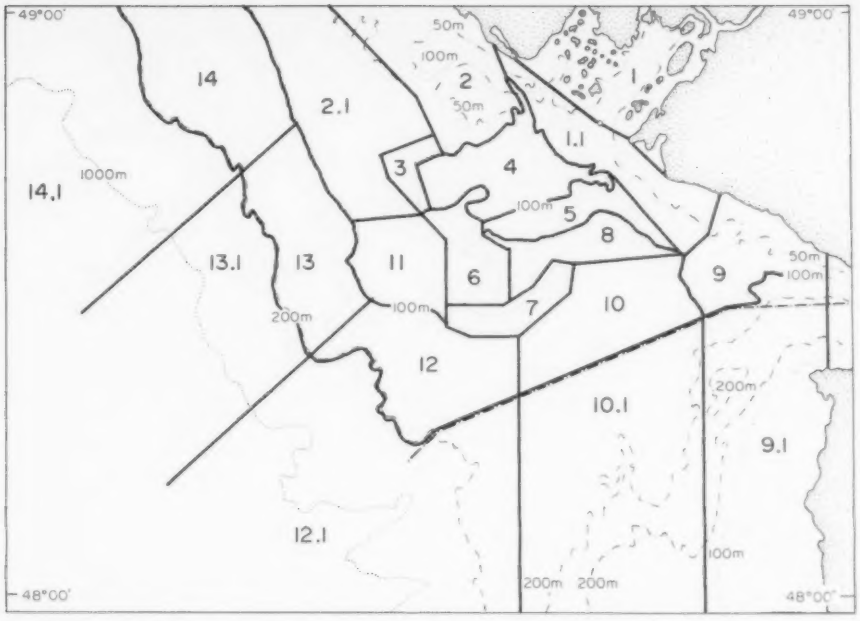

FIG. 1. Fishing subareas in the La Perouse Bank study area. The dotted line shows the Canada–U.S. border.

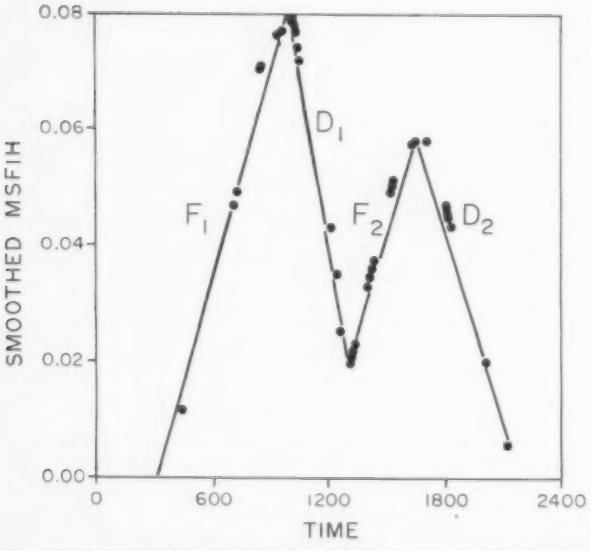

FIG. 2. Example, from hake, of trends in mean stomach fullness indices for herring (MSFIH) used for estimating daily ration. F1 and F2 are feeding periods. D1 and D2 are digestion periods. Feeding is assumed to begin at 05:30 PDT.

Daily ration was calculated using SFI trends as illustrated in Fig. 2. We assumed that a decline in smoothed MSFI over time signifies a digestive period where the digestion rate exceeds the ingestion rate. The slope of the line between the local maximum and minimum values represents the minimum evacuation rate (percent body weight evacuated per hour). The difference between the local maximum and minimum indicates the amount of prey evacuated. In contrast, increases in smoothed MSFI over time identify feeding periods, when the ingestion rate exceeds the digestion rate. We calculated the amount evacuated during each feeding period as the mean evacuation rate/2 multiplied by the duration of the feeding period. The evacuation rate was halved to reflect the lag time for the onset of digestion. Only Jones (1974) provided a quantitative estimate of the lag phase. We felt that halving the evacuation rate was the best compromise between his results and the predator and prey species we studied. Daily ration was calculated as the sum of the amount of food (percent body weight) evacuated over 1 d. Trends in the MSFI data and results presented by Livingston (1983) suggested that hake fed primarily between dawn and dusk (see Discussion). Consequently, we extrapolated trends in MSFI for hake and dogfish before calculating daily ration. (Our method is similar to the one developed by Romanov in 1958 (cited in Vinogradov 1977). Both are graphic procedures based on stomach volume. However, Romanov did not consider feeding periodicity and a lag phase.)

We adjusted ration estimates from the research surveys so they were comparable with those derived from the commercial catch data. Research surveys concentrated on sampling hake near herring concentrations and sampling all fishing subareas. In contrast, commercial samples came from the major hake concentrations and would therefore describe the feeding characteristics for the main body of hake. Data for both research and commercial fishing activities were available in August 1988 only. Smoothed MSFI values for August 1988 commercial and research data were pooled and daily ration was estimated. The ratio of the pooled estimate/research ration estimate was used to adjust all research data estimates.

We used weighted digestion codes to test our interpretation that trends in MSFI identify feeding and evacuation phases. Digestion codes for each major prey item were weighted by the

proportion of the total prey volume accounted for by that prey item in a given tow. Relationships between smoothed MSFI's and weighted mean digestion codes were tested using Kendall's coefficient of rank correlation (Wilkinson 1988).

The procedures described by Cohen (1977) were used to estimate the statistical power of analyses when required. Statistical power analysis in essence determines whether a null hypothesis cannot be rejected because it is true (in situations of high power) or because the experimental design is inadequate (low power).

Results

Hake Diet Composition

Hake diet composition is summarized in Table 1. Euphausiids and herring accounted for 93% (CV = 5.6%) of the diet for August. Euphausiids represented about 64% of the total prey volume. The euphausiid volumes were considerably less variable than herring volumes (CV = 24.2 and 55.3%, respectively). The proportion of euphausiids in the diet decreased over the summer (July–September 1988), while the proportion of herring increased.

The occurrence of herring in hake varied with month, location, and predator size over the summer of 1987. The weighted mean percentage of hake containing herring increased continuously from July through October (Table 2). Length frequency plots of herring in hake collected on the shelf and along the shelf-break are shown in Fig. 3. Results of two-way analyses of variance by month showed that the proportions of herring in each size category differed significantly between shelf and shelf-break hake in July only ($p = 0.003$). Power for insignificant tests for the other months was always less than 0.15. Changes in length distributions over time suggest a rapid offshore movement of young-of-the-year (YOY) herring beginning in August. Results of a two-way analysis of variance showed no significant effects of month or herring age-class on the proportion of shelf hake containing herring; however, power for these tests was low (<0.15). The proportion of shelf-break hake which contained herring was significantly ($p = 0.005$) lower in July, where only one size group of herring was found in the diet.

The occurrence of herring in hake stomachs increased progressively with predator size except for the smallest hake (<40 cm); these are nonmigratory fish collected only in Barkley Sound (Table 2). Heteroscedasticity precluded testing the influence of predator size on the proportion of hake containing herring. Results of simple linear regressions showed no significant influence ($p > 0.05$) of hake size on the size of ingested herring. However, the power for these tests was low (<0.62). Consequently, the data did not convincingly test the null hypothesis that there was no relationship between prey and predator size.

The proportion of hake containing herring varied considerably among years (Table 3). Four times as many hake contained herring in 1985 than in 1986. The occurrence of herring in migratory hake increased with predator size, but we could not test the significance of this effect because of heteroscedasticity. There were statistically significant regressions between ingested herring size and hake size in 1987–89, when YOY herring were prevalent (1987: $Y = 0.49X - 11.94$, $p < 0.0001$, $R^2 = 0.40$; 1988: $Y = 0.41X - 11.12$, $p < 0.0001$, $R^2 = 0.40$; 1989: $Y = 0.47X - 13.92$, $p < 0.0001$, $R^2 = 0.50$, where X = hake fork length (centimetres) and Y = herring standard length (centimetres)). The power of the 1985 (0.32) and 1986 (0.04) regressions was too low to adequately test the null hypothesis.

TABLE 1. Proportion of total stomach volume (cubic centimetres) of Pacific hake and spiny dogfish in the La Perouse Bank area accounted for by major prey items. n = number of fish examined, excluding hake with everted stomachs, H = herring, E = euphausiid, K = hake.

		Hake				Dogfish				
Interval		n	H	E	Total	n	H	E	K	Total
					Research					
Aug.-Sept.	1983	1362	0.59	0.37	0.96	0	—	—	—	—
Aug.	1985	801	0.29	0.59	0.88	528	0.04	0.86	0.04	0.94
Aug.	1986	2244	0.14	0.72	0.86	695	0.20	0.32	0.16	0.68
Aug.	1987	1698	0.23	0.71	0.94	279	0.16	0.59	0.20	0.95
Aug.	1988	3629	0.36	0.62	0.98	1073	0.16	0.40	0.34	0.90
Aug.	1989	2696	0.16	0.82	0.98	821	0.21	0.63	0.05	0.89
Mean			0.30	0.64	0.93		0.15	0.56	0.16	0.87
CV			55.3	24.2	5.6		45.1	37.8	76.8	12.7
					Commercial					
July	1987	2538	—	0.75	—	0	—	—	—	—
Aug.	1987	4953	—	0.89	—	0	—	—	—	—
Sept.	1987	5449	—	0.89	—	0	—	—	—	—
Oct.	1987	1729	—	0.76	—	0	—	—	—	—
Nov.	1987	882	—	0.45	—	0	—	—	—	—
June	1988	3157	0.01	0.91	0.92	0	—	—	—	—
July	1988	5239	0.06	0.90	0.96	0	—	—	—	—
Aug.	1988	5648	0.09	0.82	0.91	0	—	—	—	—
Sept.	1988	5419	0.17	0.69	0.86	0	—	—	—	—
Oct.	1988	690	0.10	0.52	0.62	0	—	—	—	—

TABLE 2. Percentage of hake containing herring, 1987 commercial data. Data are presented as number of fish sampled and percentage of hake sampled which contained herring. Means are weighted by the proportion of fish in each size/time combination. Fish <40 cm were collected in Barkley Sound only.

	Hake size categories (cm)							
Month	<40	40–45	46–50	51–55	56–60	61–65	>65	Mean
July	4	385	1374	641	118	14	2	
	0	0.8	0.9	3.4	7.6	21.4	0	1.9
Aug.	10	667	2391	1508	326	46	5	
	0	0.6	1.4	5.6	16.9	28.3	20.0	3.9
Sept.	14	904	2762	1411	312	41	5	
	7.1	1.2	1.6	7.6	24.4	31.7	60.0	4.7
Oct.	2	252	848	486	120	17	1	
	0	0.8	4.1	9.3	25.0	35.3	100.0	6.9
Nov.	0	81	405	276	102	15	3	
	0	0	2.5	3.3	5.9	20.0	33.3	3.3
Mean	3.3	0.9	1.7	6.2	18.0	28.6	37.5	

We found that the size of herring eaten by hake varied between years. Data from the August research surveys (Fig. 4) showed that YOY herring were absent in 1985 but accounted for a considerable proportion of the hake diet in 1988 and 1989. Analyses of variance were impossible because of heteroscedasticity.

Dogfish Diet Composition

Herring, euphausiids, and hake accounted for 87% (CV = 12.7%) of the dogfish diet (Table 1). Euphausiids made up 56% of the total prey volume, while herring and hake represented 15 and 16% of the volume, respectively. Herring, euphausiids, and hake accounted for an unusually low proportion (0.68) of the dogfish diet in 1986. The diet was more variable that year. In addition, unidentifiable digested material accounted for an unusually high proportion (0.15) of the total stomach volume. Excluding 1986, euphausiids, herring, and hake represented 92% (CV = 3.2%) of the dogfish diet.

Coefficients of variation suggested that the dogfish diet composition varied more interannually than the hake diet. Compared with hake, the euphausiid portion of the dogfish diet was more variable whereas the herring portion was less so. The hake component of the dogfish diet was extremely variable. It accounted for a minimum of 4% of the diet in 1985 and a maximum of 38% in 1988.

Consumption of herring by dogfish was influenced by year and predator size. Dogfish fed mainly on age 1 + and older fish but also took YOY fish during 1987–89 (Fig. 5). Results of an analysis of variance showed no significant influence of year and herring age-class on the proportion of dogfish containing herring; however, statistical power was less than 0.10. An analysis of variance to examine herring consumed by dogfish based on the proportion in each herring size category was impossible because of heteroscedasticity. Herring were progressively more prevalent in larger dogfish (Table 4), but we could not perform analyses of variance to test for a size effect because the variances were heterogeneous. We found no significant ($p > 0.05$) effect of predator size on prey size for any year; however, the power of any regression was low because it never exceeded 0.23.

Feeding Periodicity

Hake fed sporadically between dawn and dusk. Figure 6, based on the commercial data sets, suggested that hake begin feeding near dawn between June and September. We found no obvious trend in the number of feeding peaks or their timing. The reason for these intriguing patterns is unknown.

The dogfish data were insufficient to show when feeding began and ended. However, as in hake, there was no obvious regularity in the onset and end of feeding bouts between years.

Daily ration

We tested two assumptions before estimating the daily ration. The first assumption was that all hake samples came from one feeding aggregation, i.e. they fed with the same intensity at the same time of the day. Trends in smoothed MSFIE for various subareas in July 1988, the only period when several subareas were sampled over most of the feeding period, are shown in Fig. 7. Feeding periodicity and MSFI values were similar between shelf and shelf-break areas. We assumed this was true for dogfish also.

The second assumption we tested was that trends in SFI reflected feeding and evacuation periods, i.e. variations in SFI's did not result from randomly sampling groups of fish at various stages of energy intake. We anticipated a quadratic relationship between weighted mean digestion code and SFI. SFI should increase with digestion code as fish ingest and begin digesting food. After feeding stops, SFI should decrease as digestion code increases. Because only a few samples contained fresh material, we could only test for the negative relationship between SFI and weighted mean digestion code over the evacuation phase. Kendall coefficients of rank correlation for weighted mean digestion codes and SFI's are presented in Table 5. Thirteen of the 15 relationships were negative and four were significant ($p < 0.05$). This indicates that SFI trends do indeed reflect feeding and evacuation phases.

We used a third and untested assumption that fish rarely feed at night. In support of this assumption, we found that evacuation rates were slow enough that a substantial meal could not be taken after dusk and evacuated before dawn.

Daily rations for hake differed between and within years and between shelf and shelf-break feeding areas. Results from the adjusted August data (Table 6) showed that hake ration averages 1.60% body weight·d^{-1} (BWD). Herring accounted for a minimum and maximum of 1 and 24% of the daily ration in 1986 and 1988, respectively. The ration over the summer of 1988 averaged 1.95 and 1.27% BWD for shelf and shelf-break

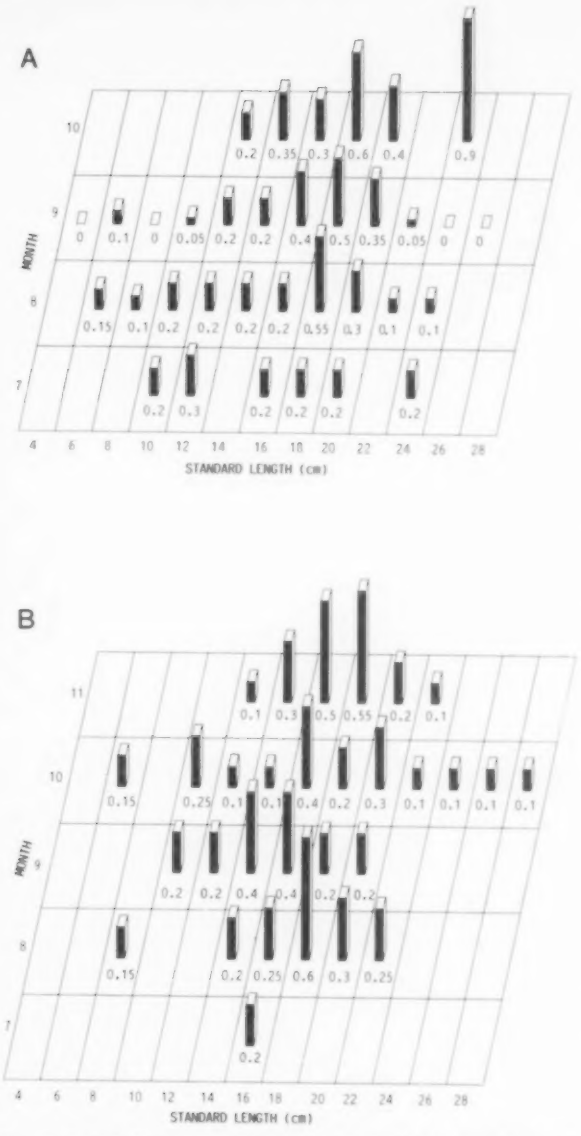

FIG. 3. Length frequency of herring in (A) shelf hake and (B) shelf-break hake collected in commercial catches, 1987. Values are mean percentages of hake containing herring of size indicated. 0 indicates $0 <$ value < 0.05.

feeding areas, respectively (Table 7). These mean summer rations were just insignificantly different ($t = 2.59$, $0.10 < p < 0.05$). The euphausiid ration was higher over the summer of 1987 in the shelf feeding area as well. The euphausiid and herring components of the diet changed in importance over the feeding season in the shelf feeding area. Euphausiid rations appeared to decline over the summer, while herring became more important in late summer.

Dogfish daily ration estimates varied with a similar magnitude as did hake ration estimates, although rations were higher (Table 8). The mean August ration over all years was 2.60% BWD.

Discussion

The diet, feeding periodicity, and daily ration of Pacific hake feeding off the lower west coast of Vancouver Island varied considerably within and between years. As with other studies (e.g. Outram and Haegele 1972; Livingston 1983), we found that euphausiids and herring were the principal prey items. However, we were also able to describe how the diet composition and daily rations change over the feeding season. The trends in the euphausiid and herring composition of the diet likely reflect seasonal and interannual changes in euphausiid biomass. We suspect that these biomass changes result, to a large extent, from hake predation.

The differences we found in ration estimates between shelf and shelf-break feeding aggregations may reflect differences in euphausiid abundance. Movements of hake, considered in the context of seasonal variations in diet composition, suggest that the main body of hake moves near Barkley Sound early in the summer to feed principally on euphausiids. Based on field observations and unpublished data, hake feed primarily on *Thysanoessa spinifera*. There is a biomass of euphausiid and fish capable of sustaining some hake offshore, but because of the consistently lower ration and spotty hake distribution, the offshore feeding environment may be inferior in early summer. The offshore area seems to become more important in late summer/fall, after euphausiid biomass on the shelf has been depleted.

We suggest that the depletion of euphausiids on the shelf by hake drives their seasonal movements in the La Perouse area. Tow positions of the commercial hake fleet (Fig. 8) indicate the location of the main body of fish. This concentration moves onshore in early summer and offshore in the autumn, and we describe a concomitant decline in the euphausiid ration for hake. Day (1971) found a marked drop in *T. spinifera* abundance off Barkley Sound between the spring and fall of 1963. We suspect that hake deplete the nearshore *T. spinifera* stock and then move offshore to locate higher densities of euphausiids. Ermakov reported in 1974 (cited in Bailey et al. 1982) that hake feeding concentrations are inshore early in the summer and move offshore in the fall. He speculated that the timing of the annual hake migration southward is in response to the seasonal appearance of the Davidson Current. This appears to be the case for migratory hake which feed in the La Perouse Bank area. Hake usually leave the area in October when, according to Thomson (1981), the Davidson Current appears. This current materializes after the "fall transition," when prevailing coastal winds shift to the southeast and consequently terminate summer upwelling conditions.

We found that hake predation on herring increased over the summer, increased with hake size, and varied interannually. This is consistent with Livingston (1983) who found that the proportion of herring increased in the diet between the spring and fall off Washington and Vancouver Island. She reported that the proportion of hake feeding on herring increased to a maximum of 30%. We also found that the incidence of herring in hake increased continuously with predator size.

The length of herring in hake stomachs varied between season and year. In part, these differences probably result from variations in abundance and movement of YOY herring out of Barkley Sound. Ware and McFarlane (1986) reported that herring year-class strength for west coast Vancouver Island stocks may be a function of hake predation.

TABLE 3. Percentage of hake containing herring, research data. Weighting and data presentation are as for Table 2. Fish <40 cm were collected in Barkley Sound only.

	Hake size categories (cm)							
Year	<40	40–45	46–50	51–55	56–60	61–65	>65	Mean
1983	213	325	438	242	76	54	14	2.9
	0	0.3	0.5	1.6	11.8	31.5	42.9	
1985	4	145	252	180	129	67	24	8.7
	0	0.7	0.8	7.7	22.5	20.9	41.7	
1986	150	540	975	434	118	19	8	2.2
	0	0.2	1.7	5.1	5.9	0	37.5	
1987	90	146	607	538	245	50	22	6.6
	2.2	4.8	3.3	5.4	14.3	28.0	22.7	
1988	278	1241	1402	483	174	35	16	5.8
	9.0	2.9	4.7	8.3	14.9	25.7	56.2	
1989	146	665	1270	489	104	17	5	2.3
	4.8	1.6	0.6	4.3	9.6	17.6	40.0	
Mean	3.9	1.8	2.3	5.5	13.7	23.6	39.3	

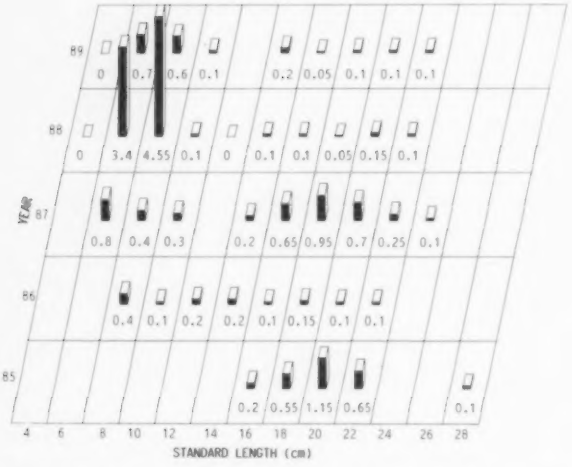

FIG. 4. Length frequency of herring in shelf hake collected in research catches.

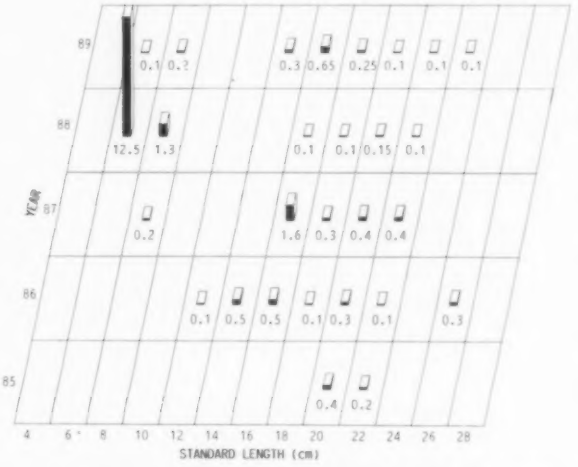

FIG. 5. Length frequency of herring in dogfish collected in research catches.

Our results suggest that herring are incidental prey. As implied by Livingston (1983), euphausiids are the single most important and unifying factor in the hake diet because they attract hake and other planktivores, like herring, which then become available prey for hake.

The dogfish diet differs somewhat from that of hake, but it also varies considerably between years. Although euphausiids are the principal prey item, dogfish feed on hake as well as herring. Fish become more important in the diet as dogfish size increases. The most interesting difference is that the offshore concentrations of dogfish do not feed on YOY herring to any extent. This is not surprising because our survey results indicate that YOY herring are not abundant where dogfish concentrate, i.e. Swiftsure Bank, located at the mouth of Juan de Fuca Strait. Jones and Geen (1977) described the diet of spiny dogfish in British Columbian waters. Herring, shrimp, euphausiids, and hake predominated. Euphausiids were the major food for young and immature dogfish throughout the year. Larger dogfish fed on euphausiids in the spring and summer and, as we found,

TABLE 4. Percentage of dogfish containing herring, research data. Weighting and data presentation are as for Table 2.

	Dogfish size categories (cm)			
Year	<60	59–79	>79	Mean
1985	86	264	178	
	0	2.3	3.4	2.3
1986	133	416	146	
	0.8	1.9	17.1	4.9
1987	16	167	96	
	12.5	7.2	27.1	14.4
1988	40	647	386	
	0	5.6	14.8	8.7
1989	211	532	78	
	1.9	3.6	12.8	4.0
Mean	1.5	4.0	14.0	

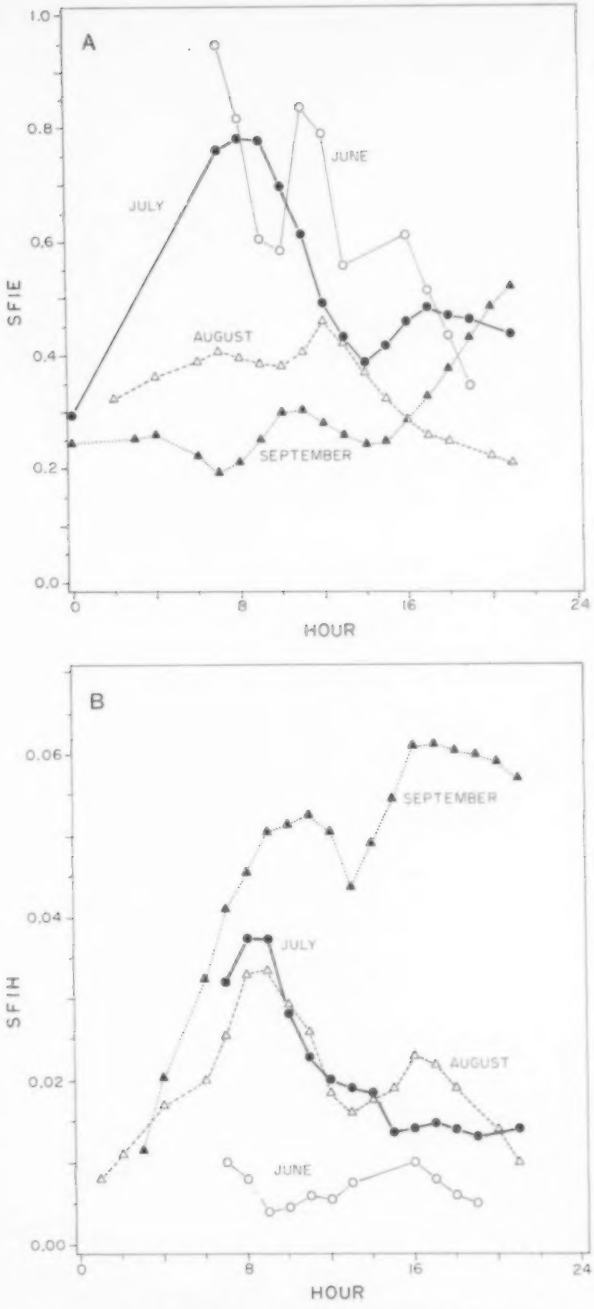

FIG. 6. Smoothed mean stomach fullness indices of (A) euphausiids (SFIE) and (B) herring (SFIH) in hake collected in commercial catches, 1988. Times of dawn and dusk are given in Table 7.

there was a transition to fish feeding with increased predator size.

Our results indicate that hake feed periodically between dawn and dusk. This is contrary to the generally accepted belief that hake feed nocturnally. Variations in SFI for our data, either observed or implied from the daily ration calculations, show

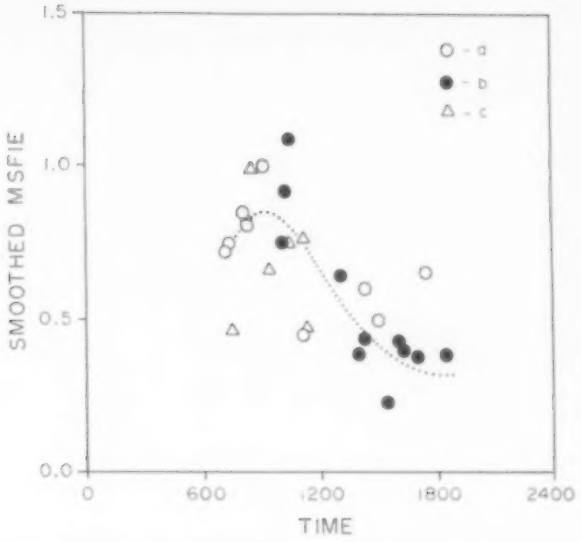

FIG. 7. Smoothed mean stomach fullness indices of euphausiids (MSFIE) in hake from various subareas, July 1988 commercial catches. a, subarea 1.1; b, subarea 4; c, subarea 12.1.

TABLE 5. Kendall coefficients of rank correlation (τ) for weighted mean digestion code and smoothed mean stomach fullness indices for herring or euphasiid in hake and for herring, euphausiids, or hake in dogfish. Data are from research cruises. $^*p < 0.05$.

	Hake		Dogfish		
Year	Herring	Euphausiid	Herring	Euphausiid	Hake
1987	−0.30	−0.68*	−0.55	−0.91	−0.33
n	15	16	4	4	4
1988	−0.03	−0.10	−0.67*	0.07	0.32
n	17	20	12	16	16
1989	−0.47	−0.67*	−0.57*	−0.16	−0.43
n	6	9	14	14	8

that feeding rarely, if ever, starts before dawn and seldom continues after dusk. All reports (Hickling 1927; Alton and Nelson 1970; Outram and Haegele 1972) describing nocturnal feeding in hake derive from studies where sampling was too infrequent to delineate the timing of feeding, or were restricted to daylight hours considerably after, or before, dawn and dusk, respectively. Our results are similar to those of Livingston (1983). She monitored hake feeding through the night and found that the SFI for hake declined steadily from 18:00 (PST) through to 00:00 and increased by 03:00. Since dawn began at 02:56 on July 15, 1967, at Seattle (C. Aikman, Dominion Astrophysical Observatory, Victoria, B.C., pers. comm.), feeding could have commenced near it.

Our empirically derived ration estimates for hake agree favourably with an indirect but independent calculation of daily ration. The daily ration over the summer of 1988 averaged 1.95 and 1.27% BWD for hake feeding on the shelf or along the shelf-break, respectively, and 1.60% BWD over all Augusts examined. These ration estimates become 2.07, 1.35, and 1.72% BWD after adjusting them upwards to account for the minor prey items in the diet. We used equations provided by Jones (1978) to estimate the amount of energy that an age 6

TABLE 6. Digestion rates (D) (% body weight·h^{-1}) and daily rations (R) (% body weight·d^{-1}) for hake collected during August research surveys. Feeding is assumed to start at dawn (04:56 PDT) and stop at dusk (21:59 PDT) unless indicated otherwise by stomach fullness index trends. Adjusted rations are calculated using the ratios of August rations estimated from commercial and research data pooled divided by rations estimated from research data only (i.e. 0.79·euphausiids, 0.28·herring). Ration estimates are for the shelf area only; the adjusted ration reflects the diet and ingestion of the main body of hake.

	Herring		Euphausiid		Daily	Adjusted ration		
Year	R	D	R	D	R	Herring	Euphausiid	Daily
1983	0.08	0.01	0.49	0.05	0.57	0.02	0.39	0.41
1985	0.59	0.03	3.57	0.19	4.16	0.17	2.82	2.99
1986	0.08	0.01	0.95	0.06	1.03	0.01	0.75	0.76
1987	0.49	0.02	2.02	0.09	2.51	0.14	1.60	1.74
1988	0.67	0.05	0.76	0.06	1.43	0.19	0.60	0.79
1989	0.38	0.06	3.55	0.34	3.93	0.11	2.80	2.91
Mean	0.39	0.03	1.89	0.13	2.28	0.11	1.49	1.60
CV	63.1	69.9	73.8	88.0	66.4	64.8	73.8	70.8

TABLE 7. Digestion rates (D) (% body weight·h^{-1}) and daily rations (R) (% body weight·d^{-1}) for hake collected from commercial catches. Feeding is assumed to start at dawn and stop at dusk. Dawn (i.e. 03:34, 03:58, 04:56, 05:50, and 06:35) and dusk (i.e. 23:15, 23:00, 21:59, 20:47, and 19:42 PDT) are for the 15th day of June through October, respectively, at Tofino, B.C.

	Shelf					Shelf-break				
	Herring		Euphausiid		Daily	Herring		Euphausiid		Daily
Month	R	D	R	D	R	R	D	R	D	R
					1987					
July	—	—	0.52	0.04	—	—	—	—	—	—
Aug.	—	—	0.66	0.05	—	—	—	0.25	0.02	—
Sept.	—	—	0.48	0.03	—	—	—	0.41	0.02	—
Oct.	—	—	0.31	0.03	—	—	—	0.14	0.05	—
Mean	—	—	0.49	0.04	—	—	—	0.27	0.03	—
CV	—	—	29.4	23.9	—	—	—	50.3	57.7	—
					1988					
June	0.04	0.01	3.84	0.24	3.88	0.16	0.02	1.98	0.19	2.14
July	0.06	0.01	1.16	0.03	1.22	0.42	0.04	0.96	0.09	1.38
Aug.	0.13	0.01	0.82	0.05	0.95	0.13	0.01	0.54	0.01	0.67
Sept.	0.35	0.02	1.39	0.11	1.74	0.13	0.01	0.76	0.05	0.89
Mean	0.15	0.01	1.80	0.11	1.95	0.21	0.02	1.06	0.06	1.27
CV	94.7	50.0	128	86.0	68.2	67.0	70.7	60.1	96.6	51.3

TABLE 8. Daily ration (R) and digestion rates (D) for dogfish collected on the shelf, August 1985–89 research data.

	Herring		Euphausiid		Hake		Total
Year	R	D	R	D	R	D	R
1985	0	—	0.78	0.10	0	—	0.78
1986	0.13	0.01	0.44	0.03	2.40	0.10	2.97
1987				Incalculable			
1988	0.15	0.01	1.62	0.09	0.81	0.06	2.58
1989	0.21	0.01	3.80	0.25	0.07	0.01	4.08
Mean	0.12	0.01	1.66	0.12	0.82	0.06	2.60
CV	73.7	0	91.0	78.0	136	75.2	52.7

hake requires for growth, maintenance, gonad growth, and swimming. We assumed that 40% of the annual growth in length occurred over the summer feeding period (Francis 1983) and that the energy density for both species of euphausiids was 1.0 kcal·g^{-1} (Mauchline 1980). We used published size-at-age data for ages 6 and 7 hake and weight–length relationships for the lower west coast of Vancouver Island (Shaw et al. 1989). Mean monthly sea temperatures at 50 m were estimated from data collected by oceanographic moorings near hake concentrations. We calculated a daily ration over the summer of 1.68% BWD for hake feeding on the shelf. This was 81% of the value we calculated from stomach content analysis (2.07% BWD).

We found that hake ration estimates obtained using Elliott and Persson's (1978) modification of Bajkov's (1935) procedure differed insignificantly from those calculated by us. We used the evacuation rate equations provided by Durbin et al. (1983) and mean August sea temperatures at 50 m for 1983 and 1985–89 to calculate hake evacuation rates. We wanted to test Jobling's (1986) suggestion that gastric emptying rates derived from single-meal experiments have limited application to field

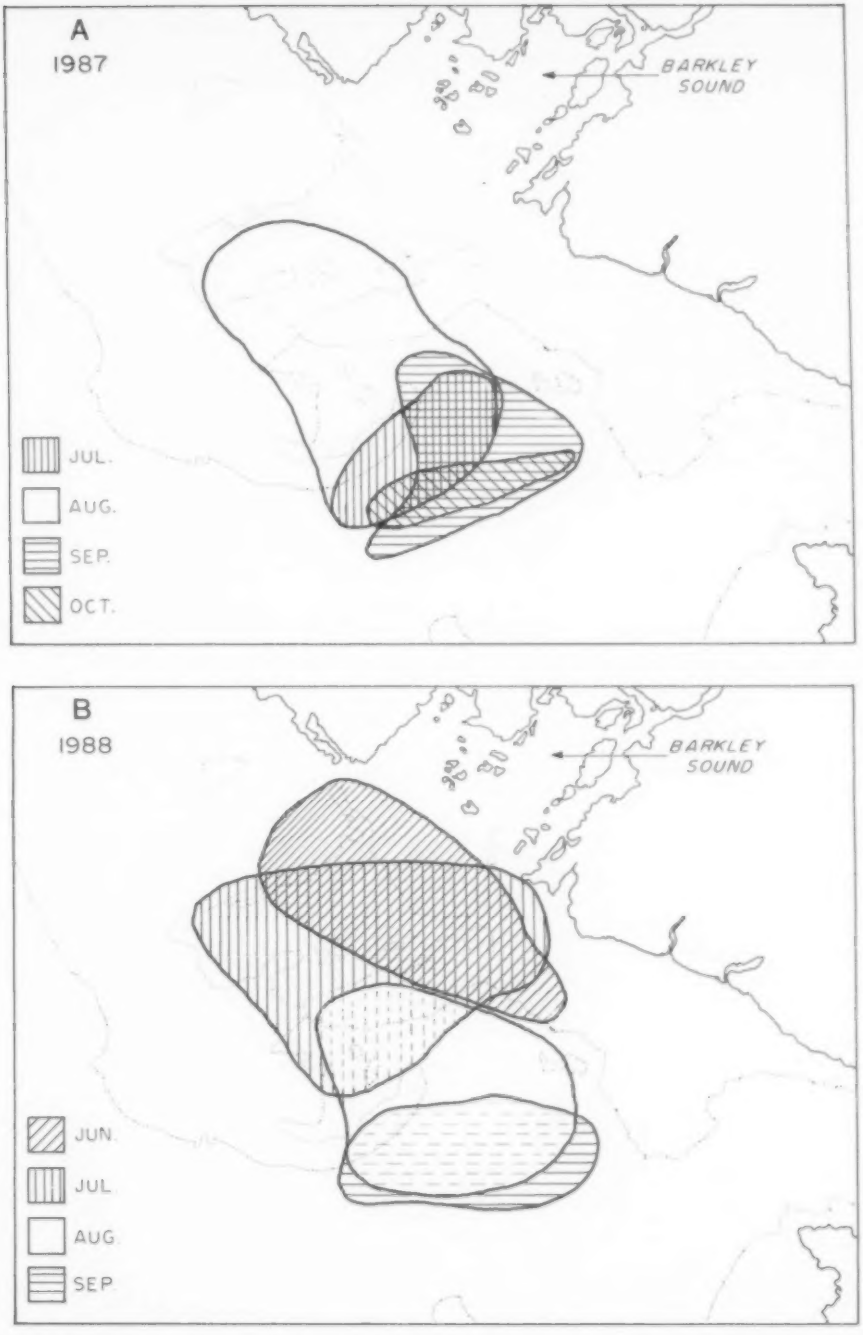

FIG. 8. Distribution of hake set locations for Canadian trawlers over the (A) 1987 and (B) 1988 fishing seasons.

studies. The resulting calculated evacuation rates for euphausiids and herring were not significantly different from those we estimated ($t < 1$ and $t = 1.62$, respectively); however, power was low (<0.11). We used the calculated evacuation rates (R) and observed MSFI's for herring and euphausiids to estimate the daily ration using the Elliott and Persson (1978) equation: $C_t = 24 \cdot \text{MSFI} \cdot R$. Calculated rations were 0.96 of those estimated using our procedure and did not differ significantly ($t = 0.22$, power = 0.70). It is encouraging that both methods give similar results. The advantage of our procedure is that it explicitly deals with feeding periodicity, whatever the pattern may be.

Ration estimates for hake on the shelf or along the shelf-break near the lower west coast of Vancouver Island are similar to those reported in other studies. Francis (1983) used a bioenergetics model and estimated a daily ration of 0.8% BWD over the growing season for 6-yr-old hake feeding off the west coast of Vancouver Island. Livingston (1983) calculated a summer daily ration of 2.5% BWD for hake off the Washington and Vancouver Island coasts. Estimates for some other gadoids average 1.14% BWD over the feeding/growth season (see Francis 1983).

Our August ration estimate for spiny dogfish (2.60% BWD) is similar to that reported by Jones and Geen (1977) and is 1.63 times that estimated by us for hake over all August sampling periods. This difference could be due to numerous factors including differences in feeding capacity or rates, assimilation efficiency, and/or the timing of growth. We found that evacuation rates of euphausiids were 1.4 times higher ($t = 6.49$, $p < 0.05$) in hake.

The trends in food evacuation for both hake and dogfish were linear for all prey items. This has implications for the digestion physiology of these species. Many authors have suggested that evacuation of prey should be a variety of time-dependent functions because of differences in prey size and composition (e.g. Jobling 1986; Persson 1986; Bromley 1987). Bromley (1987) suggested that the overall goodness-of-fit of a linear evacuation model shows that, in turbot (*Scopthalmus maximus*) at least, there is a high degree of control over the process of digestion and evacuation. This would stabilize the rate of flow of chyme into the small intestine, possibly at an optimal level for the completion of digestion and nutrient absorption. This phenomenon would also explain our observations for hake and dogfish.

Acknowledgements

We thank the captains and crews of the charter vessels *Hope Bay*, *Eastward Ho*, *Caledonian*, *Sun Maiden*, and *Ocean King* for their fishing efforts and for helping us endure the hours of nausea at sea. We thank the observers for their tenacity in sampling fish under difficult conditions. Finally, we thank Dr. Barry Smith for reviewing the manuscript.

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Assessing the Impact of Sampling Error on Model-Based Management Advice: Comparison of Equilibrium Yield per Recruit Variance Estimators

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Pelletier, D., and P. Gros. 1991. Assessing the impact of sampling error on model-based management advice: comparison of equilibrium yield per recruit variance estimators. Can. J. Fish. Aquat. Sci. 48: 2129–2139.

The assessment of fish stocks often relies on data estimated from complex sampling schemes. For instance, catch and weight-at-age estimates result from sampling commercial landings. This paper studies the propagation of sampling error in catches in an equilibrium yield per recruit model. The covariance matrix of catch estimators is calculated for a given design using sampling theory. The impact of this uncertainty on estimated yield per recruit is assessed by three techniques: the delta method, trials from a multinormal distribution of catches (Gaussian approximation), and bootstrap. The comparison of results leads to the following conclusions. (i) For the model studied, the delta method probably overestimates the variance of the response. (ii) Gaussian approximation and bootstrap give similar values. As the latter is free of approximation and of restrictive assumptions, this suggests that the yield model chosen is to some extent insensitive to the distributional form of catch estimators. (iii) Bootstrap is the method of choice, provided that resampling accurately mimics the whole complexity of the original sampling design. If resampling is not properly achieved, variances could be severely underestimated.

Les données utilisées pour l'évaluation des stocks de poissons sont souvent estimées à partir de procédures d'échantillonnage complexes. Ainsi, les estimations de captures et de poids aux âges proviennent de l'échantillonnage des captures commerciales. Cet article s'intéresse à la propagation de l'erreur d'échantillonnage des captures dans un modèle de rendement par recrue à l'équilibre. La matrice de covariance des estimateurs des captures est calculée pour un protocole donné par application de la théorie de l'échantillonnage. L'impact de cette incertitude sur l'estimation du rendement par recrue est évalué grâce à trois techniques : la delta-méthode, des tirages selon une distribution multinormale pour les captures (approximation Gaussienne) et le bootstrap. La comparaison des résultats amène aux conclusions suivantes : i) Dans le cas du modèle étudié, la delta-méthode sur-estime probablement la variance de la réponse. ii) L'approximation Gaussienne et le bootstrap donnent des valeurs semblables. Ce dernier ne requérant pas d'approximation ni d'hypothèse restrictive, cela suggère que le modèle de production est dans une certaine mesure insensible à la forme de la distribution des estimateurs des captures. iii) Le bootstrap est la méthode à choisir, pourvu que le rééchantillonnage reproduise précisément toute la complexité de la procédure d'échantillonnage; les variances pourraient sinon être gravement sous-estimées.

Received July 25, 1990
Accepted May 9, 1991
(JA661)

Reçu le 25 juillet 1990
Accepté le 9 mai 1991

Many fish stocks are assessed with the help of diagnostic and prognostic models. Model parameters are often estimated from Virtual Population Analysis (VPA) (Gulland 1965) which reconstructs population sizes and fishing intensity. Catches in numbers are the main input to VPA. Thus, the quality of these data finally determines the relevance of management decisions inferred from assessment results. The absolute dependence of these results on catch data is characterized by the sensitivity of models to catch estimates (Pelletier 1990). Catches in numbers are estimated from the sampling of commercial landings. The uncertainties arising from such estimations, e.g. the covariance matrix of catch estimators, are highly dependent on sampling design. The sampling programs are often complex, including stratification and multistage procedures. Particularly, clustering modifies sampling variances and covariances of estimated variables (Kish and Frankel 1974). Thus, the sample covariance matrix of catches must be carefully evaluated in connection with sampling design.

The impact of sampling error on management decisions may be assessed by studying the propagation of this matrix through the models used. This is achievable in different ways. For instance, the delta method has been applied in several cases (Rivard 1981; Sampson 1987; Prager and MacCall 1988; Pelletier 1990). Also, simulations using a Gaussian distribution have been explored (Pope and Gray 1983; Rivard 1983). The consequences of the first-order approximation inherent to the delta method have to be evaluated. This may be accomplished by comparing the results of the delta method with those of simulations which allow higher-order effects to be taken into account. However, the implications of the assumption of a Gaussian law for sampling distribution of catches must also be checked. This could be done by reproducing the sampling pro-

cedure without an a priori distributional hypothesis (nonparametric simulation). Resampling techniques like bootstrap (Efron 1979) are then useful.

This paper studies the propagation of sampling error in catches through a VPA connected with an equilibrium yield per recruit model. First, the covariance matrix of catch estimators is computed analytically, taking into consideration the sampling design. Then, the propagation of this sampling error is assessed from three techniques: the delta method, Gaussian approximation, and bootstrap. The three approaches are then compared in terms of required initial assumptions, types of results, and probable accuracy of variance estimators. Finally, some conclusions are drawn concerning possible improvements of inferences on model outputs within the framework of stock assessment.

Material and Methods

Long-Term Equilibrium Yield per Recruit Model

Yield per recruit is a classical diagnostic tool for management advice, especially in regulating fishing effort, e.g. mesh size or fishing intensity. The total yield produced by a group of recruits entering the fishery is computable by integrating all the catches in weight over the fishable life span of these recruits. The exploitation pattern is characterized by a vector $\boldsymbol{F}$ of mortality rates F_a for each age-group a. If this exploitation pattern and the annual recruitment remain unchanged for some years, at least during the average lifetime of a cohort, both the stock and the fishery reach an equilibrium, and the annual yield per recruit Y_r is constant and equal to the annual fish weight produced per young recruit. Following Beverton and Holt (1957), equilibrium yield per recruit is expressed, using conventional notation, as

$$(1)\quad Y_r = \sum_{a=1}^{A-1} \frac{F_a W_a S_a}{Z_a}(1-\exp(-Z_a)) + \frac{F_A W_A S_A}{Z_A}$$

where a indexes age-group and W_a is the mean weight at age a in the stock (fish growth during the year is therefore neglected and changes in average weight due to age selectivity of fishing mortality are ignored), Z_a is the overall mortality rate given by $Z_a = F_a + M_a$, S_a is the proportion of survivors with respect to knife-edge recruitment:

$$S_a = \exp\left(-\sum_{j=1}^{a-1} Z_j\right)$$

and A relates to a "plus-group" corresponding to fishes of age A and older, i.e. there are no survivors in this group.

Yield per recruit can be calculated for a range of exploitation patterns, and comparison with a reference allows one to assess the long-term impacts of changes in fishing effort. New patterns $\boldsymbol{F}$ are derived from the reference fishing mortality vector noted $\boldsymbol{F}_{\text{ref}}$ as follows:

$$(2)\quad \boldsymbol{F} = \mu\,\boldsymbol{F}_{\text{ref}}.$$

Hence, the corresponding yield per recruit may be seen as a function $Y_r(\mu, \boldsymbol{F}_{\text{ref}}, \boldsymbol{W}, \boldsymbol{M})$. In this paper, weights and natural mortality are assumed to be known without error, μ is a controlled parameter, and $\boldsymbol{F}_{\text{ref}}$ is estimated by fitting a VPA model to catch-at-age data. For the sake of simplicity, shorthand notations, $Y_r(\mu, \boldsymbol{F}_{\text{ref}})$ or $Y_r(\mu)$ will be used hereafter.

Description of the Stock Studied

The stock under study is the Celtic Sea Cod stock, harvested in ICES quarters 7f, g, h, and j. It is worth noting the prevalence of French landings (Anonymous 1989), almost 90% of total landings on average. Moreover, the stock is exploited by one fleet of trawlers mainly coming from Lorient, a southern Brittany harbour where the procedure for sampling catches is precisely defined (see below). Recruitment in this fishery occurs at age 1 and there are seven age-groups, the last one being a plus-group. Concerning model inputs, data estimated for 1988 are used, i.e. for each age-group, catches in numbers, mean weights, terminal fishing mortality, and natural mortality (Anonymous 1989).

Data Sampling and Estimation of Fishing Mortality Rates

As we assume that weights and natural mortality are fixed quantities, age-specific catches are the data of interest in this study. The catches are estimated from sampling of commercial landings in the harbour previously mentioned. These estimates are in fair agreement with those from the working group (Anonymous 1989).

In the context of sampling theory, the landings from a given stock represent a statistical population. The sampling design involves several sampling units (Fig. 1). Landings are first stratified according to market-size categories. Each of the H strata comprises N_h days which are primary units of unequal size because the amount of landings varies from day to day. Boxes are secondary units of sizes M_{hi} which are assumed to have the same weight (50 kg). Thus, in each stratum h, the sampling scheme is a three-stage procedure: first drawing without replacement of n_h days among N_h days; for each day i previously sampled, second drawing without replacement of m_{hi} boxes among M_{hi} boxes; in each box j previously sampled, simple random sampling (SRS) of fishes with rate f_h, depending on market category h, i.e. in order to allow proportional allocation.

The basic information obtained from the sampling units at the third stage is then the number of cods of age a, noted $\hat{nc}_{hija}$. The variance of catch-at-age estimators has three components: between-days term; between-boxes term; within-box term.

Consistent with the SRS, the within-box term corresponds to a multinomial model. Following classical sampling theory (Cochran 1977; Kendall et al. 1983), the analytical expression of catch estimators together with the estimator of their covariance matrix is derived and reported in Appendix 1. An unbiased estimator was chosen whereas its variance is theoretically greater.

Indeed, there is a simplification in the previous description of the sampling procedure: a first sample is drawn in each box sampled in order to estimate catches-at-length. Then, a second sample is drawn from each class of length to estimate proportions of catches-at-age per length, i.e. the age–length key. Hence, stratification according to length within a box is neglected. However, as stratification does not generate variance, and is on the contrary intended to reduce it, this omission likely results in a slight overestimation of variance.

Catch-at-age is an important input to VPA. VPA serves to estimate the fishing mortality rates F_a required for the computation of equilibrium yield per recruit $Y_r(\mu, \boldsymbol{F}_{\text{ref}})$ (see VPA equations in Appendix 2). The present analysis is intended for a single pseudocohort. Actually, the catch-at-age vector used, namely the vector of estimates for year 1988, is not represent-

ative of a single cohort. But, under the equilibrium assumption required for yield per recruit, it is equivalent to consider either the fishing mortality vector of a given cohort or the fishing mortalities of the different cohorts of the stock at a given year. Using the 1988 catch vector within the VPA model therefore produces fishing mortalities F_a that are not contradictory for equilibrium computations.

Variance of Yield per Recruit Estimators

The relevance of management policy closely relies on the accuracy of the advisory tools. Therefore, it is of prime importance to assess the impact of sampling error on the response of the yield per recruit model. Accuracy is characterized by the variance of $\hat{Y}_r(\mu, \hat{F}_{ref})$. For this purpose, three techniques will be used: resampling (bootstrap), delta method (taylorization), and Gaussian approximation.

Bootstrap

The basic principle of bootstrap (Efron 1979, 1982) is to replicate the reference catch-at-age vector in order to estimate yield per recruit for each replicated sample. Given a series of such estimates $\hat{Y}_r^*$, the variance $\hat{V}^*(\hat{Y}^*_r)$ is computed over all values. Conceptually, the bootstrap algorithm aims at reproducing sampling variability. Thus, the way bootstrap samples are replicated must be consistent with the original sampling design giving rise to the reference sample (Efron and Tibshirani 1986). In other words, bootstrap resamples according to the empirical distribution reflected by the initial sample. For instance, if the reference sample is a simple random one, the stochastic generating process is a multinomial law with parameters p_a, i.e. proportions of fish of age a in the population of landings. The reference sample allows estimation of these parameters as

$$(3)\quad \hat{p}_a = \frac{\hat{C}_a}{\hat{C}} \quad \text{where} \quad \hat{C} = \sum_a^A \hat{C}_a.$$

In this case, bootstrap replicates are drawn following the multinomial distribution $M(\hat{C},(\hat{p}_a),\ a=1...A)$. However, such a simple resampling scheme assumes the equiprobability of selection of every fish in the landings. This is obviously not the case for the sampling scheme previously described. The primary units, i.e. days, have unequal sizes, since the quantity of landings is variable from day to day. Hence, a given fish does not have the same probability of being drawn on every day.

The simulation of a complex sampling design implies a detailed knowledge of the procedure used, e.g. strata sizes, the number of primary and secondary units, their age composition. The second stage corresponding to boxes is neglected here to simplify the replication process. Finally, the bootstrap algorithm mimics both the stratification of landing days by market category and quarter, and the following two-stage sampling scheme is adopted. In each stratum h: drawing with replacement of n_h days among the n_h days already drawn in the initial sample (days are equiprobable); and for each day previously drawn, drawing with replacement of size $nc_{hi} = \sum_a^A nc_{hia}$ with probabilities $\hat{p}_{hia} = nc_{hia}/nc_{hi}$ according to a multinomial model.

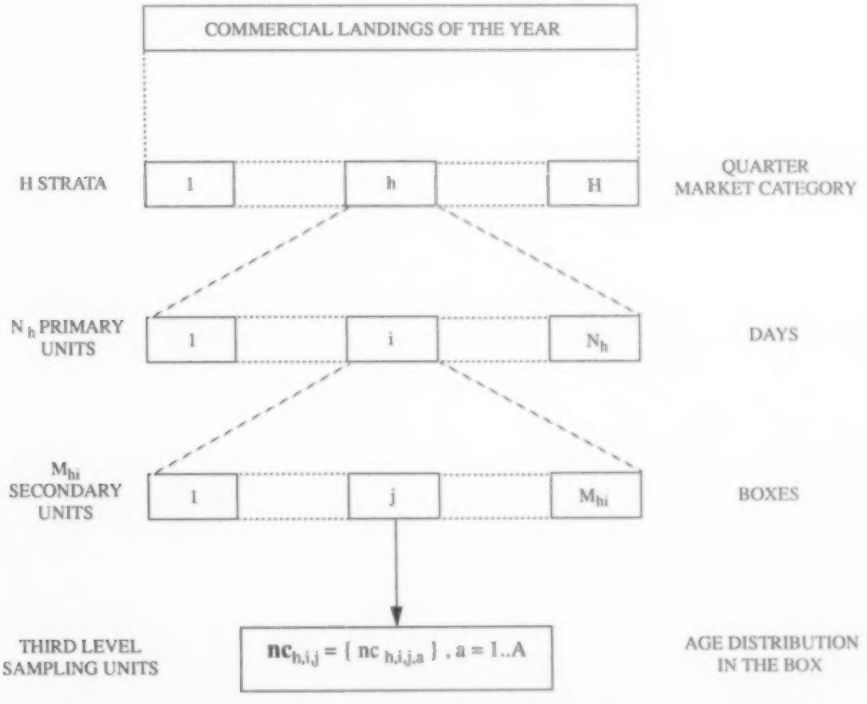

FIG. 1. Partition of the population of commercial landings according to sampling design. Each stratum corresponds to a market category of a given quarter. The sizes of strata, primary, and secondary units are known.

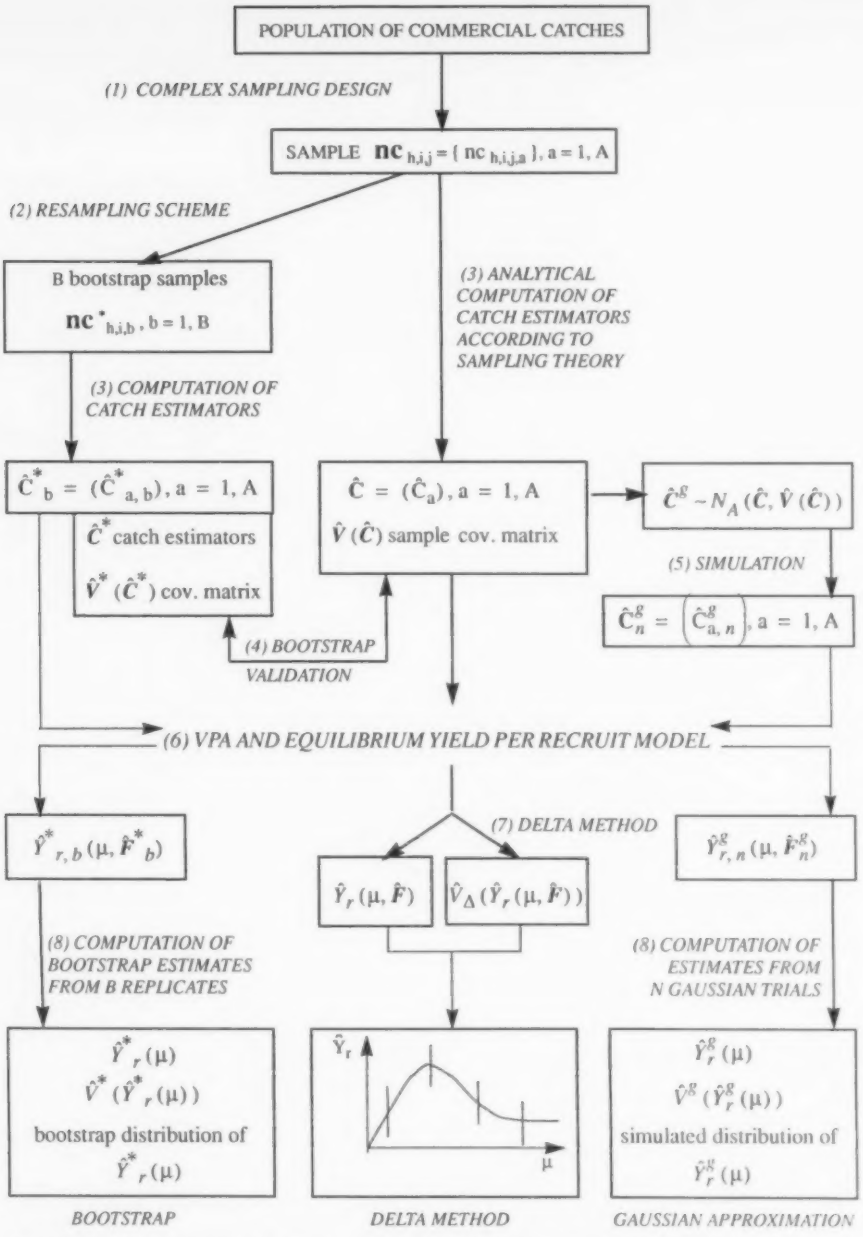
POPULATION OF COMMERCIAL CATCHES
(1) COMPLEX SAMPLING DESIGN
SAMPLE nc h,i,j = { nc h,i,j,a }, a = 1, A
(2) RESAMPLING SCHEME
B bootstrap samples
nc * h,i,b , b = 1, B
(3) ANALYTICAL COMPUTATION OF CATCH ESTIMATORS ACCORDING TO SAMPLING THEORY
(3) COMPUTATION OF CATCH ESTIMATORS
Ĉ* b = (Ĉ* a, b), a = 1, A
Ĉ* catch estimators
V̂* (Ĉ*) cov. matrix
Ĉ = (Ĉa), a = 1, A
V̂ (Ĉ) sample cov. matrix
Ĉg ~ NA (Ĉ, V̂ (Ĉ))
(5) SIMULATION
Ĉg n = (Ĉg a, n), a = 1, A
(4) BOOTSTRAP VALIDATION
(6) VPA AND EQUILIBRIUM YIELD PER RECRUIT MODEL
(7) DELTA METHOD
Ŷ* r, b (μ, F̂* b)
Ŷr (μ, F̂)
V̂Δ (Ŷr (μ, F̂))
Ŷg r, n (μ, F̂g n)
(8) COMPUTATION OF BOOTSTRAP ESTIMATES FROM B REPLICATES
(8) COMPUTATION OF ESTIMATES FROM N GAUSSIAN TRIALS
Ŷ* r (μ)
V̂* (Ŷ* r (μ))
bootstrap distribution of
Ŷ* r (μ)
Ŷr
μ
Ŷg r (μ)
V̂g (Ŷg r (μ))
simulated distribution of
Ŷg r (μ)
BOOTSTRAP
DELTA METHOD
GAUSSIAN APPROXIMATION

FIG. 2. Flowchart of computations performed in the three techniques used for estimating yield per recruit variance: bootstrap, the delta method, and assumption of a multinormal sampling distribution of catch-at-age estimators. From top to bottom: (1) Complex sampling design of landings; subscript h, i, and j: index strata, days, and boxes, respectively. (2) Resampling scheme which mimics previous design except for boxes, data being aggregated over boxes. This resampling provides B bootstrap samples. (3) Computation of catch-at-age estimators, either from initial sample ($\hat{C}$, middle) or from each bootstrap sample ($\hat{C}_b^*$, left). Notice the validation step (4) which compares bootstrap estimates C^* and $\hat{V}^*(\hat{C}^*)$ with the results from standard analysis $\hat{C}$ and $\hat{V}(\hat{C})$. These latter estimates define parameters for the multinormal model assumed in the parametric simulation (5) which generates N trials $\hat{C}_g$. Whatever their origin (bootstrap replicates, classical sample statistics, or Gaussian trials), catch estimates are processed through VPA and resulting $\hat{F}$ enters equilibrium yield per recruit model (6). The variance of model response $\hat{Y}_r(\mu, \hat{F})$ is then computed (7 and 8) according to the technique of variance estimation. Note that the delta method (7) only provides standard error estimation whereas bootstrap and Gaussian approximation yield in addition an estimation of $Y_r(\mu, \hat{F})$ distribution.

TABLE 1. Covariance matrix of catches, as estimated following classical analysis. Values are percent coefficients of variation and covariation.

Age	1	2	3	4	5	6	7+
1	39	0	0	0	0	0	0
2		23	3	1	0	0	0
3			21	13	0	0	0
4				21	11	20	14
5					52	20	−11
6						53	25
7+							65

TABLE 2. Components (expressed in percentages) of the sample variance of catch-at-age estimators. The last column shows percent proportions of each age-group in the total catch in weight. Notice that groups 2 and 3 contribute to 80% of landings; associated variance mainly originates from between-days heterogeneity.

Age	Between days	Between boxes	Within boxes	Proportion of catches
1	100	0	0	6
2	99.99	1×10^{-4}	0	67
3	87.4	11.8	8×10^{-3}	11
4	70.6	26.9	2.5	9
5	74.9	21.7	3.4	4
6	93.9	3.5	2.5	2
7+	7	92.9	0.1	1

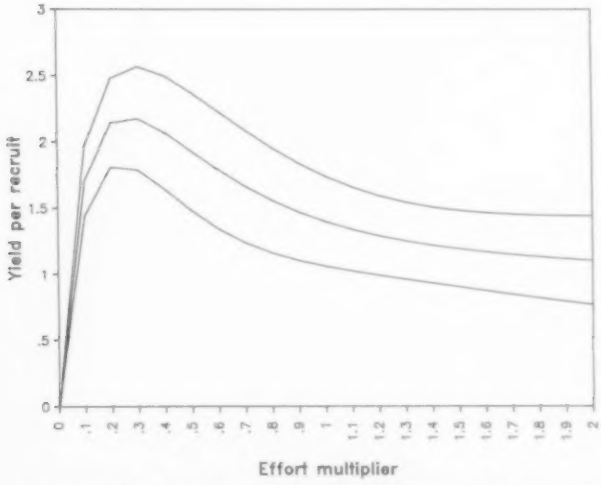

FIG. 3. Yield per recruit versus effort multiplier μ. The upper and lower curves represent yield plus or minus two standard deviations estimated from the delta method.

Taylorization

Expression of yield per recruit described in equation (1) is expandable at first-order in Taylor series. This approximation leads to a variance estimator given in Appendix 3. The taylorization or delta method (Cramér 1961) requires an estimation of input parameter covariance matrix. The output variance is then expressed in an analytical way as a function of input variances and covariances. In this approach, the estimation is obtained at first-order and the yield is expressed directly as a function of catch, not of fishing mortalities. An alternative may be to approximate yield variance from fishing mortalities variances, which themselves could be approximated from catch variances. In that case, the first-order approximation would be applied twice and the estimation of yield variance would probably be less reliable.

TABLE 3. Covariance matrix for catches as estimated from bootstrap. Values are percent coefficients of variation and covariation.

Age	1	2	3	4	5	6	7+
1	33	−4	−4	5	8	−3	−8
2		20	−2	−2	−6	−5	6
3			23	9	4	−2	−8
4				25	16	15	8
5					50	33	8
6						59	0
7+							100

TABLE 4. Mean catch-at-age estimated for each age-group in numbers ($\times10^3$ individuals).

	Age						
	1	2	3	4	5	6	7+
Analytical	1013	4245	234	113	42	17	4
Bootstrap	938	3911	220	108	39	17	4

TABLE 5. Percent coefficients of variation of catches estimated by a "naïve" bootstrap from a multinomial model.

	Age						
	1	2	3	4	5	6	7+
Naïve bootstrap	6	2	5	11	18	22	37

Gaussian approximation

In this analysis, a third technique for variance estimation is also tried. It relies on the assumption that the sample distribution of catch-at-age estimators is multinormal. The expected vector and covariance matrix of this distribution are estimated from sampled data. Despite its close similarity with the so-called parametric bootstrap (Efron 1982), this technique will be named "Gaussian approximation" hereafter. In parametric bootstrap, the normality assumption (for example) would concern basic disaggregated data and not catch estimators which are a function of these data.

In this application, the delta method and the Gaussian approximation both use catch estimators and the estimator of their covariance matrix previously computed from sampling theory (see Appendix 1). A flowchart illustrates the main stages of this analysis, as well as inputs and outputs (Fig. 2). Simulation-based techniques such as bootstrap and the Gaussian approximation require a large number of iterations. Two thousand replicates have been used in this application in order to estimate empirical distributions.

Results

Sample Variance of Catch-at-Age Estimators

Applying formulae from Appendix 1 to cod data for the year 1988 yields an estimation of covariance matrix (Table 1). Relative precision decreases sharply for the extreme age-groups because they are seldomly found in catches. Zero between-boxes variance is obtained for the first and second age-groups

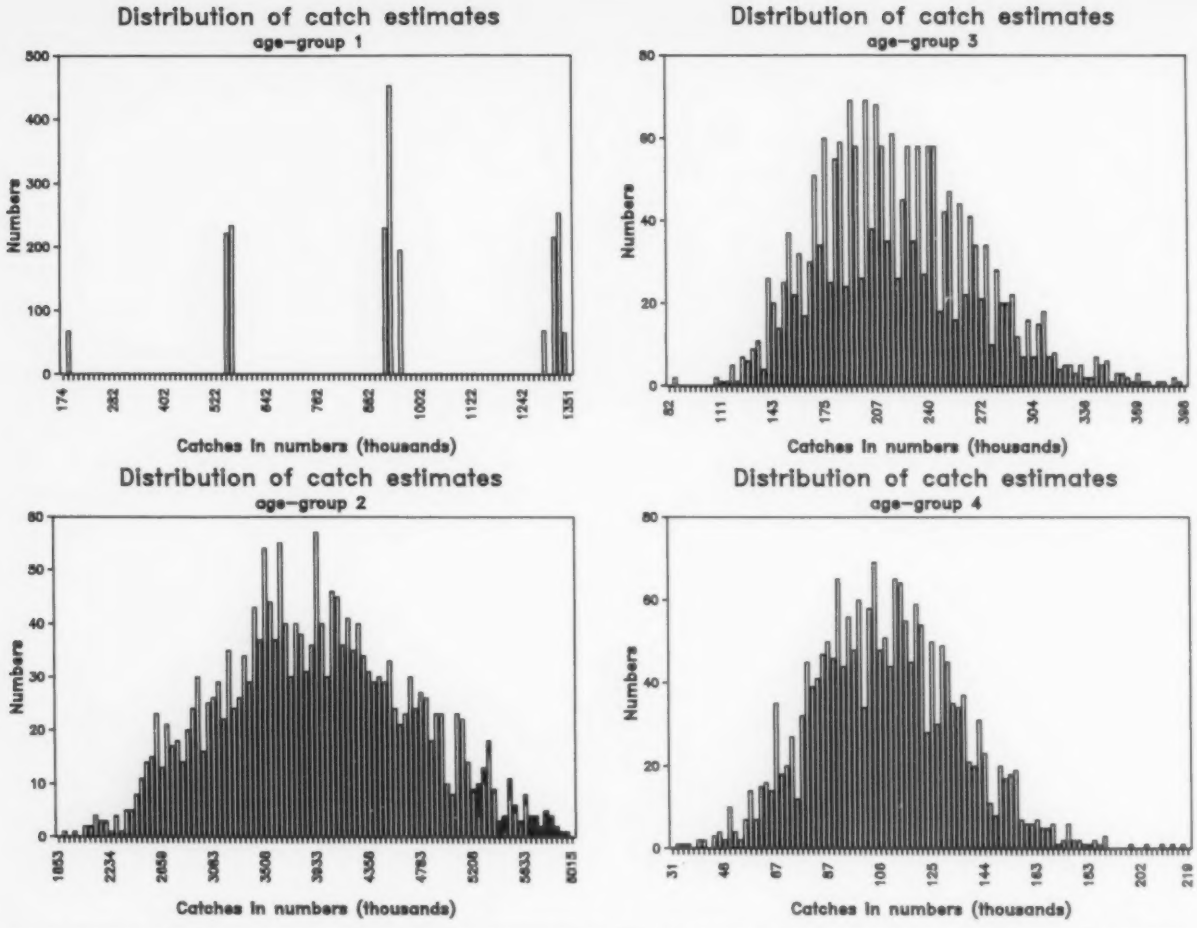

FIG. 4. Bootstrap distributions of catch-at-age replicates for economically important age-groups. The distributions are obtained from 2000 replicates.

TABLE 6. Coefficients of variation of yields per recruit estimated from the delta method, bootstrap, and Gaussian approximation, respectively.

Effort multiplier	Delta method	Bootstrap	Gaussian approximation
0.1	8	5	6
0.2	8	4	4
0.3	9	4	4
0.4	10	4	4
0.5	12	5	5
0.6	12	5	5
0.7	13	5	5
0.8	13	5	5
0.9	13	5	5
1	12	5	5
1.1	12	5	5
1.2	12	5	5
1.3	12	5	5
1.4	12	5	5
1.5	12	5	5
1.6	13	5	5
1.7	13	5	5
1.8	14	5	6
1.9	15	5	6
2	15	6	6

because only one box per day is sampled in corresponding market categories. This results in a reduction of corresponding total variances and enhances the discrepancy between the variances for old and young age-groups. Variance may be split into components according to the stages of the sampling scheme (Table 2). The proportion of catch (by weight) stresses the relative importance of each age-group in landings. The intrabox component, which corresponds to the SRS of third stage units and therefore accounts for a multinomial distribution, is obviously negligible. In contrast, between-days variance is preponderant, except for last age-group, reflecting the strong variability of landings over time. Most covariances between age-groups are positive. A multinomial model, which implies negative covariances by definition, is thus obviously inappropriate for reproducing the data covariance structure in a complex design because of multiple stages that generate large variance components, e.g. between-days variance in this example. More generally, in multistage sampling schemes, the leading variance component is the between-primary units term (Kendall et al. 1983).

First-Order Error Propagation

Consistent with the model description in the first section, yields per recruit $\hat{Y}_r(\mu)$ are computed for a range of effort mul-

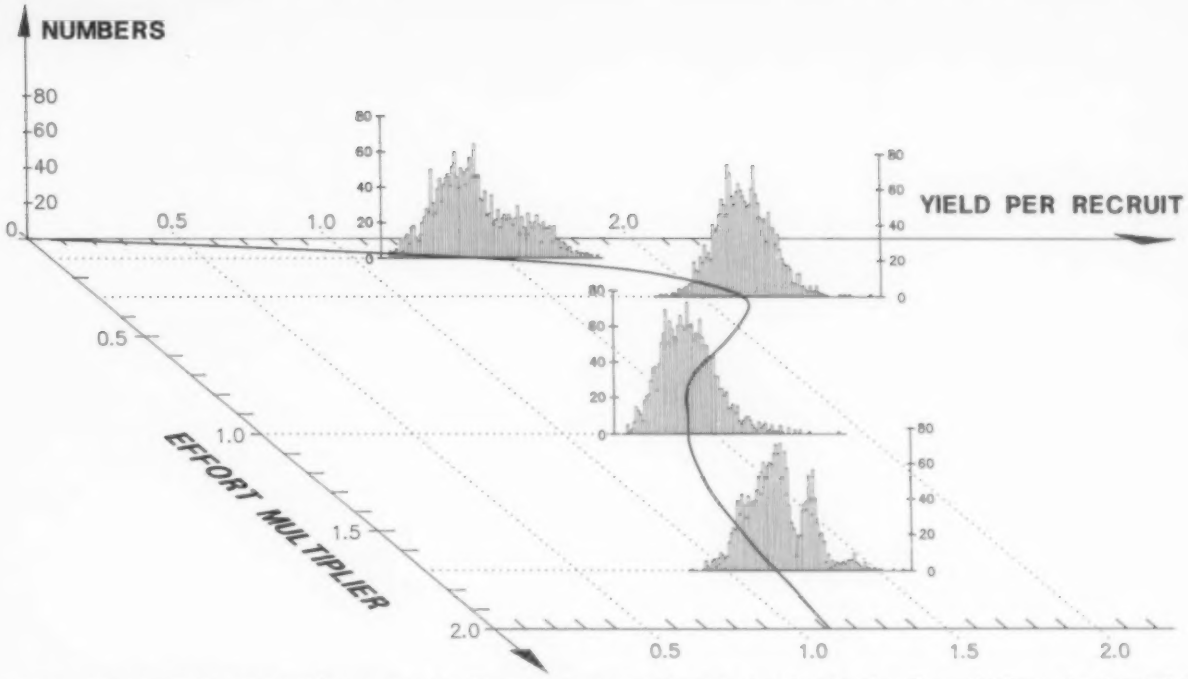

FIG. 5. Three-dimensional picture of bootstrap results. The yield per recruit estimate is plotted against the effort multiplier on the plane $\langle xOy \rangle$. Four bootstrap distributions are pictured, corresponding to values of effort multiplier 0.1, 0.3, 1, and 1.7.

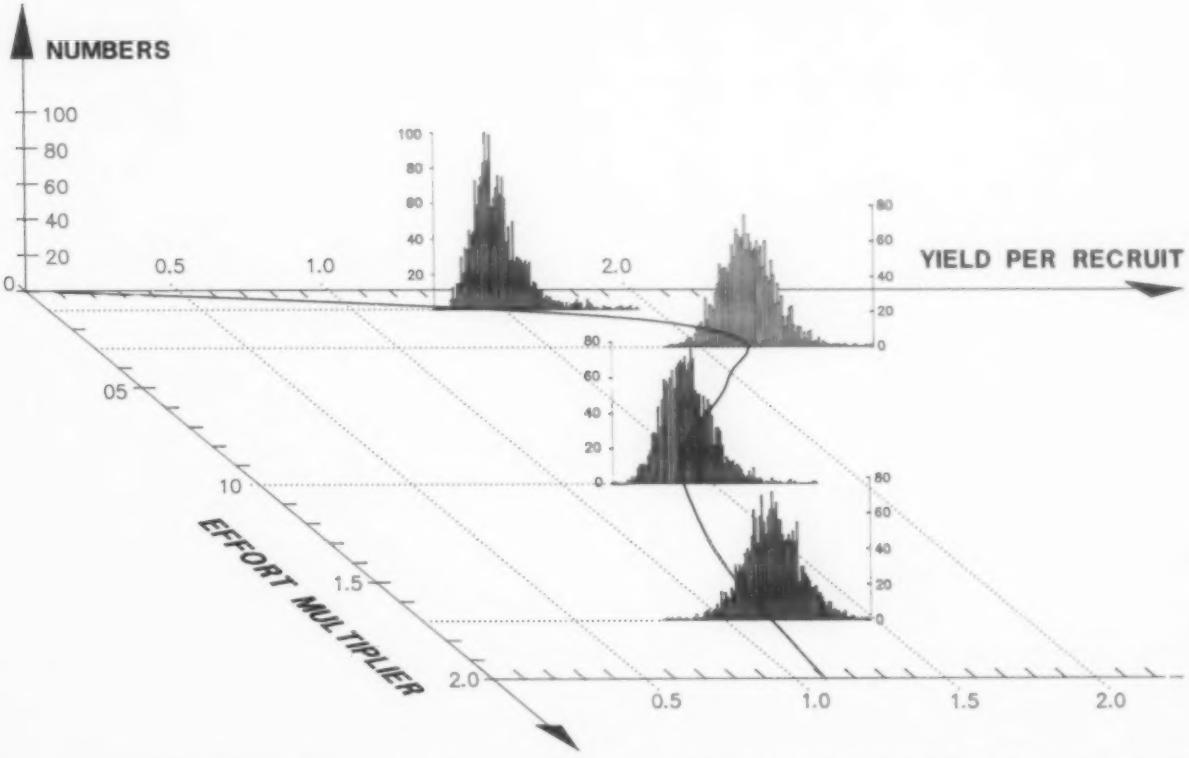

FIG. 6. Three-dimensional picture of results obtained from Gaussian trials. The yield per recruit estimate is plotted against the effort multiplier on the plane $\langle xOy \rangle$. Four simulated distributions are pictured, corresponding to values of effort multiplier 0.1, 0.3, 1, and 1.7.

tipliers μ from 0 to 2 (Fig. 3). Upper and lower curves represent the estimation plus or minus two standard deviations, respectively:

$$\hat{Y}_r \pm 2\sqrt{\hat{V}_\Delta(\hat{Y}_r(\mu))}.$$

Final results, i.e. yields, are emphasized, rather than intermediate VPA results, i.e. fishing mortalities. As was stressed in a previous work (Pelletier 1990), yield per recruit is less sensitive to catches than VPA results. Hence, the coefficients of variation of fishing mortalities are approximately equal to those of catch estimators whereas yield variance is lower than input catch-at-age errors. Consequently, the uncertainty due to catches is moderate. Finally, coefficients of variation (CVs) of yield range between 8 and 15%. A comparison with the other two methods is carried out hereafter.

Simulation-Based Approaches of Error Propagation

Bootstrap and Gaussian approximation are both based on replication of catch vector according to a predefined procedure. Let us first consider the bootstrap results. Resampling is achieved by the stratified two-stage design described in Material and Methods. Recall that the second stage of the original sampling scheme corresponding to boxes has been neglected owing to the weak between-boxes variance component (see Table 2: the component is nonnegligible only for age-groups 4, 5, and 7+, whose contribution to landings is less than 15%). Resampling is applied to disaggregated cod data which have been used to estimate the covariance matrix (Table 1). The bootstrap process validation is performed by comparing catch-at-age and covariance matrix estimates obtained by analytical computation (Table 1) with those from bootstrap (Tables 3 and 4). The methods show a fair agreement between the catch-at-age estimates (maximum relative discrepancy of 7%). Bootstrap estimates are in general lower than analytical ones. Regarding relative variances and covariances, estimates are also quite close. Some discrepancies exist for old age-groups which are economically unimportant. Several covariance terms appear to be nonzero, although they relate to strata sampled independently. This is possibly an artifact of the simulation: two variables may be numerically correlated, even in absence of any causal relationship. In any case, these terms are small compared with others, and probably not statistically significant. On the whole, the results from both methods compare satisfactorily, and the resampling is thus validated for assessing sampling error propagation. As a comparison, a "naïve" bootstrap, i.e. assuming a SRS of catches, was also tried (Table 5). The CVs decrease as the number of catches for each age-group increases. Thus, they are very small for economically important ages. Obviously, such a bootstrap severely underestimates variances because it neglects the complexity of sampling design, and particularly multiple stages which generate variance.

Bootstrap also provides distributions of catch estimators (Fig. 4). Distributions appear rather symmetrical for age-groups prevalent in landings, but they are skewed and even multimodal for other age-groups, e.g. age 1. The null hypothesis of a Gaussian approximation is rejected for all age-groups when tested.

Once the resampling procedure has been validated, estimates of yield per recruit variance may be considered. Whatever the effort multiplier, bootstrap provides lower CV values than the delta method (Table 6). Besides, the bootstrap is more informative than the analytical approach: bootstrap provides the distribution of yield per recruit replicates in addition to mean values and variance estimates (Fig. 2). Such distributions allow calculation of nonparametric confidence intervals (Efron 1981, 1987). Bimodal distributions appear for extreme multiplier values (Fig. 5). Regarding the F_{max} exploitation level, namely around $\mu = 0.3$ (see Fig. 3), bootstrap distribution is unimodal. If a progressive regulation of fishing effort is pursued, for instance when a stock is overexploited, effort multipliers close to 1 are of more interest. In this case, distributions are unimodal too and appear to have a log-normal form, although this was rejected statistically. Occurrence of bimodal yield distributions for extreme multiplier values may be connected with an increased dependence of corresponding yields on age-groups whose catch distribution is itself bimodal or very skewed (see for instance distributions of catch for age-group 1 and yield for multiplier 1.7, Fig. 4 and 5).

Consider now the assessment of error propagation from parametric simulations. Gaussian trials also yield means, variances, and distributions (Fig. 6). Coefficients of variation of yields are very close to bootstrap estimates (Table 6).

Discussion and Conclusion

Sample Covariance Matrix of Catch Estimators

The covariance matrix of catch estimators has been estimated according to the sampling theory. In a similar context, Gavaris and Gavaris (1983) also noticed the sharp decrease of precision for first age-group and old age-groups. They found lower CVs (at least twice as low) using a double sampling scheme along with the delta method to approximate catch variances. Their approach relied on the estimation of an age–length key whose statistical error was assessed by Southward (1976) and Kimura (1977). In the present study, catches were estimated without an age–length key, providing unbiased estimators, but of variance theoretically greater (Cochran 1977). Using the unbiased estimator, Quinn II et al. (1983) confirmed a slight increase of variance in a case of sampling catches, but it was not sufficient to account for the discrepancy with the results in Table 1. This difference is first likely to be inherent in the three-stage sampling design which generates variance components. Second, the results cited above refer to very different stock situations.

Comparison of Techniques for Error Propagation Analysis

As quoted in Efron (1981), the delta method and bootstrap derive from the same basic idea: the observed data are held fixed, and the standard error of the quantity of interest is estimated from the variation in evaluations performed at reweighted versions of the empirical probability distribution. According to the choice of the resampling vectors, other techniques are defined in the same way, e.g. the jackknife. The idea underlying the Gaussian approximation is similar, but in a parametric context. Despite their theoretical close connection, all three methods used in this study differed in their practical consequences. This point is examined now to decide which method can be chosen to provide the most reliable estimator.

Required assumptions

The hypotheses in each approach are considered below, from the most to the less constraining framework.

Concerning Gaussian approximation, the sampling distribution of catches is supposed to be multinormal. In the case studied, such an assumption is obviously wrong (see Fig. 4).

However, the question is not only about its validity, but about model robustness with respect to departure from this hypothesis. Are model results much different whether this assumption is violated or not? Present results suggest that variance estimates from bootstrap and Gaussian approximation are quite close. With another catch vector, bootstrap variances were a little lower than Gaussian approximation variances (not reported here).

The delta method applied at first-order approximates yield per recruit as a linear function of catches. Hence, the variance formula is only valid for small catch variances. Higher-order effects resulting from large errors are thus ignored. Furthermore, the expected response of the model is assumed equal to the one obtained when using the mean values of catch-at-age, i.e. bias is neglected. Most models of concern in fisheries science and more generally in population dynamics are highly nonlinear. Examining for didactic purpose a simple but highly nonlinear function, Gardner et al. (1981) emphasized the rapid deterioration of linear approximation when the CV of the parameters increases above 30%. In the present study, the CVs of catches range between 20 and 65%. However, economically important age-groups exhibit values around 20%. The delta method is thus mainly distinguishable from simulation-based techniques, i.e. bootstrap and Gaussian approximation, by the simplification of the model resulting from first-order Taylor expansion.

Bootstrap implementation is not subject to any hypothesis. Basically, the sampling distribution of catch estimates is derived from the information given by sampled data and sampling design. At this point, it is worth emphasizing the crucial dependence of bootstrap on the sampling design, which establishes the link between the random variables of interest (i.e. catch estimators) and the unknown probability model. The information used to produce resamples relates to the process generating the data, and to the observations themselves: no additional assumption is required. As a consequence, if both samples and resamples are not properly obtained, bootstrap produces meaningless results.

Reliability of variance estimators

Different techniques for variance estimation lead to different kinds of results and by-products (Fig. 2). Strictly speaking, the delta method applied at first-order only yields variance estimates. The construction of confidence intervals requires additional knowledge about distribution of yield estimators. Actually, the upper and lower curves in Fig. 3 are not described as 95% confidence bounds, which assume a Gaussian distribution for yield estimators. Besides, the simulation-based techniques provide the same series of results: variances and empirical distributions of yield estimators.

In the above results, the delta method yields the highest CVs whereas the two simulation-based techniques yield similar results. This observed discrepancy may be attributed to the first-order approximation. However, a discrepancy between bootstrap and Gaussian approximation results is liable to occur in some cases. Indeed, the three techniques are to be compared more basically.

On the one hand, bootstrap resamples disaggregated data whereas on the other hand, these observations are pooled into an expectation vector and an initial estimation of covariance matrix, which are used once in the other two techniques. These statistics are jointly sufficient in the case of a multinormal distribution (Kendall and Stuart 1979), and the gap between the delta method and Gaussian approximation thus illustrates the effects of first-order approximation. Moreover, when bootstrap and Gaussian approximation are not equivalent (i.e. outside the multinormal framework), the discrepancy between results is then attributable to the information loss occurring when data are summarized by $\hat{C}$ and $\hat{V}(\hat{C})$.

Note that the different variance estimates may be compared, but cannot be located with respect to the true error matrix which is unknown. In the present analysis, however, the resampling scheme and therefore bootstrap results have been validated with respect to initial catch estimators and their covariance matrix. Consequently, bootstrap results are likely to be the closest to reality. Tests performed on artificial data sets, for instance, may support this hypothesis.

The Problem of VPA Calibration

In practice, VPA models are often calibrated with respect to ancillary data to improve results, e.g. terminal fishing mortality estimate. In this study, however, the VPA prior to yield calculation is not calibrated. Actually, as the delta method requires the calculation of derivatives, it cannot be used in calibration which implies an iterative process. Unless model results are expressed in a simpler way (Pope 1983), simulation remains the only solution. As catches are also used during the tuning phase, it must be stressed that the model sensitivity to catches changes depending on whether calibration is used or not. Other simulation results carried out with artificial data (not reported here) indicate that yield variances with respect to catches are lower in calibration by an ad hoc technique (Pope and Shepherd 1985).

Recommendations

When processing data collected according to a well-defined random sampling scheme, bootstrap outperforms Gaussian approximation and the delta method for three reasons: it does not require a priori assumptions about the sampling distribution of input parameter estimators; it does not require approximation of the model of concern; and it yields estimates of the sampling distribution of catch estimators which can stress particular features as skewness, or the existence of several modes.

In the present study, the resampling procedure has been validated with respect to classical estimates from sampling theory. It must be emphasized that resampling should correctly mimic the way data were collected. For instance, simulating a multinomial model when data are not obtained from SRS gives seriously misleading results. In particular, it leads to severe underestimation of variances if the original sampling design is a multistage one.

However, bootstrap may be difficult to implement when data originate from several sources, as it is usually the case in stock assessment. In these conditions, the resampling procedure becomes more complex. When bootstrap is not applicable for whatever reason, variance of model outputs should be approximated from parametric simulation, at least for models resembling the case studied. These models appear to be insensitive to the assumption of normality for catch distribution.

Extension to Other Parameters and Models

Regarding yield per recruit model parameters, the present analysis was restricted to catches. In practice, however, catches are not the only error-prone input to predictive assessment models. Other data originating from complex designs (sam-

pling of commercial landings and scientific surveys) may be processed in the same way, e.g. mean weights-at-age or abundance indices. Weights are an important input for predictions because yield is linear with respect to them (Rivard 1981; Pelletier 1990).

Concerning other models, catch projections represent a crucial step in an assessment. The catch projections model relies on an equation analogous to (1) and depends moreover on recruitment. Conclusions about equilibrium yield per recruit broaden to short-term catch projections in that both models behave similarly relative to common parameters. Furthermore, the present simulations could be extended to abundance indices used for VPA calibration and recruitment estimation.

Acknowledgements

We thank D. Rivard for reviewing an earlier draft of the paper. Anonymous referees are also gratefully acknowledged.

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Appendix 1: Estimators of Catch-at-Age from Sampling Theory

Let $\hat{nc}_{ahij}$ be the estimated number of catches of age-group a in box j, day i, and stratum h. Following the notations of Fig. 1, total catch for age a is then estimated by

$$\hat{C}_a = \sum_h^H \frac{N_h}{n_h}\left(\sum_{i=1}^{n_h}\frac{M_{hi}}{m_{hi}}\sum_j^{m_{hi}}\hat{nc}_{ahij}\right).$$

The variance of catch estimator for age a is estimated by

$$\hat{Var}(\hat{C}_a) = \sum_{h=1}^{H}\left\{\frac{(N_h-n_h)N_h}{n_h(n_h-1)}\sum_i^{n_h}(\hat{nc}_{ahij}-\hat{nc}_{ah})^2\right\}$$

$$+\sum_{h=1}^{H}\left\{\left(\frac{N_h}{n_h}\right)\sum_i^{n_h}\frac{M_{hi}(M_{hi}-m_{hi})}{(m_{hi}-1)m_{hi}}\sum_{j=1}^{m_{hi}}(\hat{nc}_{ahij}-\hat{nc}_{ahi})^2\right\}$$

$$+\sum_{h=1}^{H}\left\{\left(\frac{N_h}{n_h}\right)\left(\frac{1-f_h}{f_h}\right)\sum_i^{n_h}\left(\frac{M_{hi}}{m_{hi}}\right)\sum_{j=1}^{m_{hi}}\frac{nc_{ahij}(\hat{nc}_{hij}-\hat{nc}_{ahij})}{\hat{nc}_{hij}}\right\}.$$

Similarly, covariances between estimators for age a_1 and a_2 are estimated by

$$\hat{Cov}(\hat{C}_{a1},\hat{C}_{a2}) = \sum_{h=1}^{H}\left\{\frac{(N_h-n_h)N_h}{n_h(n_h-1)}\times\sum_i^{n_h}(\hat{nc}_{a1hi}-\hat{nc}_{a1h})(\hat{nc}_{a2hi}-\hat{nc}_{a2h})\right\}$$

$$+\sum_{h=1}^{H}\left\{\left(\frac{N_h}{n_h}\right)\sum_i^{n_h}\frac{M_{hi}(M_{hi}-m_{hi})}{(m_{hi}-1)m_{hi}}\times\sum_{j=1}^{m_{hi}}(\hat{nc}_{a1hij}-\hat{nc}_{a1hi})(\hat{nc}_{a2hij}-\hat{nc}_{a2hi})\right\}$$

$$-\sum_{h=1}^{H}\left\{\left(\frac{N_h}{n_h}\right)\left(\frac{1-f_h}{f_h}\right)\sum_i^{n_h}\left(\frac{M_{hi}}{m_{hi}}\right)\sum_{j=1}^{m_{hi}}\frac{\hat{nc}_{a1hij}\hat{nc}_{a2hij}}{\hat{nc}_{hij}}\right\}.$$

Appendix 2: Estimation of Fishing Mortalities

The VPA model helps to estimate fishing mortalities used in the yield per recruit model. Basic coupled equations (Gulland 1965) are the survival equation

$$N_a = N_{a+1}\exp(-(F_a+M_a))$$

and the catch equation

$$C_a = N_{a+1}\frac{F_a}{F_a+M_a}(\exp(F_a+M_a)-1)$$

where notations stand for age-group a, $a = 1,\ldots A$: C_a = catches in number, N_a = cohort size in number, F_a = fishing mortality rate in year^{-1}, and M_a = natural mortality rate in year^{-1}.

The catch equation is modified when the last age-group A is a plus-group:

$$C_A = N_A\frac{F_A}{F_A+M_A}.$$

Appendix 3: Delta Methods Approximation of Yield per Recruit Variance

First-order Taylor expansion of Y_r (equation (1)) yields the following formula for its variance with respect to catches:

$$\hat{V}_\Delta(Y_r) = \sum_a \left(\frac{\partial Y_r}{\partial C_a}\right)^2 \hat{\text{Var}}(\hat{C}_a) + \sum_{a\neq b}\left(\frac{\partial Y_r}{\partial C_a}\right)\left(\frac{\partial Y_r}{\partial C_b}\right)\hat{\text{Cov}}(\hat{C}_a,\hat{C}_b).$$

Partial derivatives are evaluated at the true values of catches. As noted in the first section, yield per recruit may be considered as a function $Y_r(\mu, \boldsymbol{F}_{\text{ref}}, \boldsymbol{W}, \boldsymbol{M})$ where Fishing mortality vector $\boldsymbol{F}_{\text{ref}}$ depends on catch vector estimate $\hat{\boldsymbol{C}}$. Let us write yield per recruit as follows to compute its partial derivatives with respect to catches:

$$Y_r(\mu,\boldsymbol{F}_{\text{ref}},\boldsymbol{W},\boldsymbol{M}) = \sum_{a=1}^{A} W_a Y_a(\mu,\boldsymbol{F}_{\text{ref}},\boldsymbol{W},\boldsymbol{M})$$

where $\forall\, a < A$:

$$Y_a = \frac{F_a S_a}{Z_a}(1-\exp(-Z_a))$$

and

$$Y_A = \frac{F_A S_A}{Z_A}.$$

Derivatives of Y_a are themselves computable from F_a derivatives. For $a < A$, let

$$G_a = \frac{1}{F_a} - \frac{1}{Z_a} + \frac{1}{\exp(Z_a)-1}$$

then

$$\frac{\partial F_a}{\partial C_a} = \frac{1}{C_a G_a}$$

$$\frac{\partial F_a}{\partial C_{a+1}} = -\frac{1}{C_{a+1}G_{a+1}G_a}$$

$$\forall b>a+1,\ \frac{\partial F_a}{\partial C_b} = \frac{\partial F_a}{\partial C_{a+1}}\prod_{k=a+1}^{b-1}\left(1-\frac{1}{G_k}\right)$$

$$\frac{\partial F_A}{\partial C_a} = 0.$$

Derivatives of Y_a, are then deduced as

$$\forall b,\ \forall a<A-1,\ \frac{\partial \ln Y_a}{\partial C_b} = \frac{\partial F_a}{\partial C_b}\left(\frac{1}{F_a}-\frac{\mu}{Z_a}+\frac{\mu}{\exp(Z_a)-1}\right) - \mu\sum_{k=1}^{a-1}\frac{\partial F_k}{\partial C_b}$$

$$\forall b,\ \frac{\partial \ln Y_A}{\partial C_b} = -\mu\sum_{b=1}^{A-1}\frac{\partial F_b}{\partial C_b}.$$

Lastly, partial derivatives of Y_r with respect to catches are computed from

$$\forall b,\ \frac{\partial Y_r}{\partial C_b} = \sum_{a=1}^{A} W_a Y_a \frac{\partial \ln Y_a}{\partial C_b}.$$

Meristic and Morphometric Variation between the Endangered Acadian Whitefish, *Coregonus huntsmani*, and the Lake Whitefish, *Coregonus clupeaformis*, in the Canadian Maritime Provinces and the State of Maine, USA

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Edge, T. A., D. E. McAllister, and S. U. Qadri. 1991. Meristic and morphometric variation between the endangered Acadian whitefish, *Coregonus huntsmani*, and the lake whitefish, *Coregonus clupeaformis*, in the Canadian Maritime Provinces and the State of Maine, USA. Can. J. Fish. Aquat. Sci. 48: 2140–2151.

Principal component and discriminant function analyses of morphological variation between whitefish populations from the Canadian Maritime Provinces and the State of Maine, USA, clearly distinguished the Acadian whitefish, *Coregonus huntsmani*, from the lake whitefish species complex, *Coregonus clupeaformis*. Ten meristic and 44 morphometric characters were examined and the species were best discriminated by number of vertebrae (>97% separation) and mouth shape. Acadian whitefish had a terminal mouth and 64–67 ($\bar{X}$ = 65.3) vertebrae, while lake whitefish had a subterminal mouth and 58–64 ($\bar{X}$ = 60.6) vertebrae. Acadian whitefish were also characterized by having more lateral line scales (88–100, $\bar{X}$ = 93.8) than lake whitefish (63–95, $\bar{X}$ = 76.6), a shorter adipose to caudal fin length, a smaller adipose fin, and a longer pelvic axillary process than lake whitefish. Considerable morphological variation was found between lake whitefish populations examined although there was no morphological basis to recognize any lake whitefish population as taxonomically distinct. The Acadian whitefish has morphological characteristics of both subgenera *Leucichthys* and *Coregonus*; it could be important for understanding the evolution of coregonine fishes and its imminent extinction would represent a serious loss of genetic diversity.

Des analyses par composantes principales et fonction discriminante des variations morphologiques entre les populations de corégones des provinces Maritimes et de l'État du Maine (É.-U.) permettent clairement de distinguer le corégone d'Acadie, *Coregonus huntsmani*, du complexe spécifique du grand corégone, *Coregonus clupeaformis*. On a examiné 10 caractères méristiques et 44 caractères morphométriques : les espèces se distinguent le mieux par le nombre de vertèbres (>97 % de séparation) et la forme de la bouche. Le corégone d'Acadie a une bouche terminale et 64–67 ($\bar{X}$ = 65,3) vertèbres tandis que le grand corégone a une bouche subterminale et de 58–64 ($\bar{X}$ = 60,6) vertèbres. Le corégone d'Acadie se caractérise également par un plus grand nombre d'écailles sur la ligne latérale (88–100, $\bar{X}$ = 93,8) que le corégone de lac (63–95, $\bar{X}$ = 76,6), une distance plus courte entre les nageoires adipeuse et caudale, une nageoire adipeuse plus petite et un processus axilaire pelvien plus long que le grand corégone. La variation morphologique est considérable entre les populations de grand corégone examinées, bien qu'il n'y ait pas de base morphologique pour distinguer taxinomiquement quelque population de grand corégone que ce soit. Le corégone d'Acadie présente des caractéristiques morphologiques des deux sous-genres *Leucichthys* et *Coregonus*; il pourrait apporter beaucoup à la compréhension de l'évolution des corégones et sa disparition représenterait une perte importante de diversité génétique.

Received June 19, 1990
Accepted May 9, 1991
(JA619)

Reçu le 19 juin 1990
Accepté le 9 mai 1991

The phenotypic plasticity of whitefishes within the genus *Coregonus* has presented problems for systematists since the time of Linnaeus. As a result of the difficulty of recognizing species within the genus, the Acadian whitefish,[1] *Coregonus huntsmani*, went undiscovered for many years. The species appears to have been first reported by Huntsman (1922). He mentioned that two whitefish specimens, doubtfully identified as *Coregonus quadrilateralis* (=*Prosopium cylindraceum*), were caught at the mouth of the Sissiboo River in southwestern Nova Scotia in September 1919. These specimens now appear likely to have been Acadian whitefish (Scott and Scott 1988). Subsequently, the Acadian whitefish was reported as a variable form of the Sault whitefish, *Coregonus labradoricus*

[1]We follow McAllister (1990) and Legendre (1978) in using the more geographically precise name Acadian rather than Atlantic.

(=*C. clupeaformis*), from Milipsigate Lake, NS., by Piers (1927). The Acadian whitefish was not recognized as a new species until reported by Leim and Scott (1966). The species was initially described as *Coregonus canadensis* by Scott (1967); however, this species name was found to be preoccupied and the replacement name *C. huntsmani* was proposed by Scott (1987).

The Acadian whitefish is endemic to Nova Scotia and is known only from three landlocked lakes in the Petite Rivière watershed and from anadromous populations in the lower Petite Rivière and Tusket River watersheds (Edge 1984, 1987). The construction of dams interfering with migration, overfishing, and the acidification of its habitat have reduced population levels of the Acadian whitefish to the point where the species faces extinction (McAllister et al. 1985; Edge 1987). The Acadian whitefish was classified as an endangered species by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) in 1983.

The Acadian whitefish can be readily distinguished from most North American whitefish species within the genus *Coregonus* (Scott and Crossman 1973). However, the distinction between the Acadian whitefish and the lake whitefish species

TABLE 1. Collection data for whitefish specimens examined in morphological studies.

Code	Locality	Number	Capture date	Catalogue No.
		Coregonus huntsmani		
Z	Tusket River, Yarmouth Co., N.S.	1	Aug. 31, 1954	ROM 16676
Z		1	1954	ROM 16276
Z		4	May 7, 1964	ROM 22911
Z		1	Apr. 10, 1951	ROM 15074
Z		1	Oct. 21, 1954	ROM 16672
Z		1	Nov. 1, 1954	ROM 16673
Z		1	Nov. 4, 1954	ROM 16674
Z		1	Oct. 27, 1954	ROM 16675
Z	Annis River, Yarmouth Co., N.S.	1	Oct. 11, 1982	NMC82-684
Z		1	Oct. 12, 1982	NMC82-689
Z	Yarmouth Harbour, N.S.	1	June 12, 1940	ROM 13181
U	Hall's Harbour, Kings Co., N.S.	1	May 31, 1958	ROM 19446
A	Hebb Lake, Lunenburg, Co., N.S.	6	Nov. 12, 1982	NMC82-805-7
A		4	May 24, 1983	NMC83-188
A		3	July 12, 1983	NMC84-1456
A	Milipsigate Lake, N.S.	5	Aug. 1955	ROM 17658-62
A		9	Sept. 1982	NMC82-614-23
A		2	Nov. 1982	NMC82-789
A	Minamkeak Lake, N.S.	5	Nov. 9, 1982	NMC82-802-3
Total		49		
		Coregonus clupeaformis		
G	Lake George, Yarmouth Co., N.S.	21	Oct. 7, 1982	NMC82-664-7
G		1	Sept. 25, 1983	NMC84-1454A
H	Caribou Lake, Lunenburg Co., N.S.	1	Sept. 27, 1982	NMC82-632
H	Little Mushamush Lake, N.S.	2	Sept. 28, 1982	NMC82-638
F	Annis Lake, N.S.	1	—	ROM 23296
P	Pringle Lake, Guysborough Co., N.S.	7	July 27, 1973	ROM 29118
L	Salmon River, Cape Breton Co., N.S.	5	June 22, 1982	ROM 40367
M	Mira River, N.S.	14	Jan. 1983	NMC84-1409-15
M		25	July 5, 1983	NMC84-1409-15
D	Grand Lake, N.B.	12	Nov. 16, 1958	ROM 21677
J	Saint John River, N.B.	20	May 20, 1970	—
Y		1	—	USNM-45459
X		1	Aug. 28, 1957	ROM 18829
I	Black's Harbour, N.B.	1	—	ROM 19447
K	Kerr Lake, N.B.	1	July 3, 1945	ROM 13854
K		22	July 13, 1950	ROM 25331
R	Trouser's Lake, N.B.	3	May 17, 1964	ROM 22844
B	Baker Lake, N.B.	3	Sept. 1942	ROM 13440-2
N	Third Lake, N.B.	26	June 20, 1939	ROM 10851-2
Q	Squatook Lake, Temiscouata Co., Que.	1	July 1893	USNM 45457
T	Lake Temiscouata, Que.	26	Oct. 1951	NMC 71-79
E		6	Nov. 13, 1980	NMC 80-1205
S	Square Lake, ME	23	Nov. 10, 1903	USNM 88414
O	Long Pond, ME	12	1958	ROM 23769
Total		211		

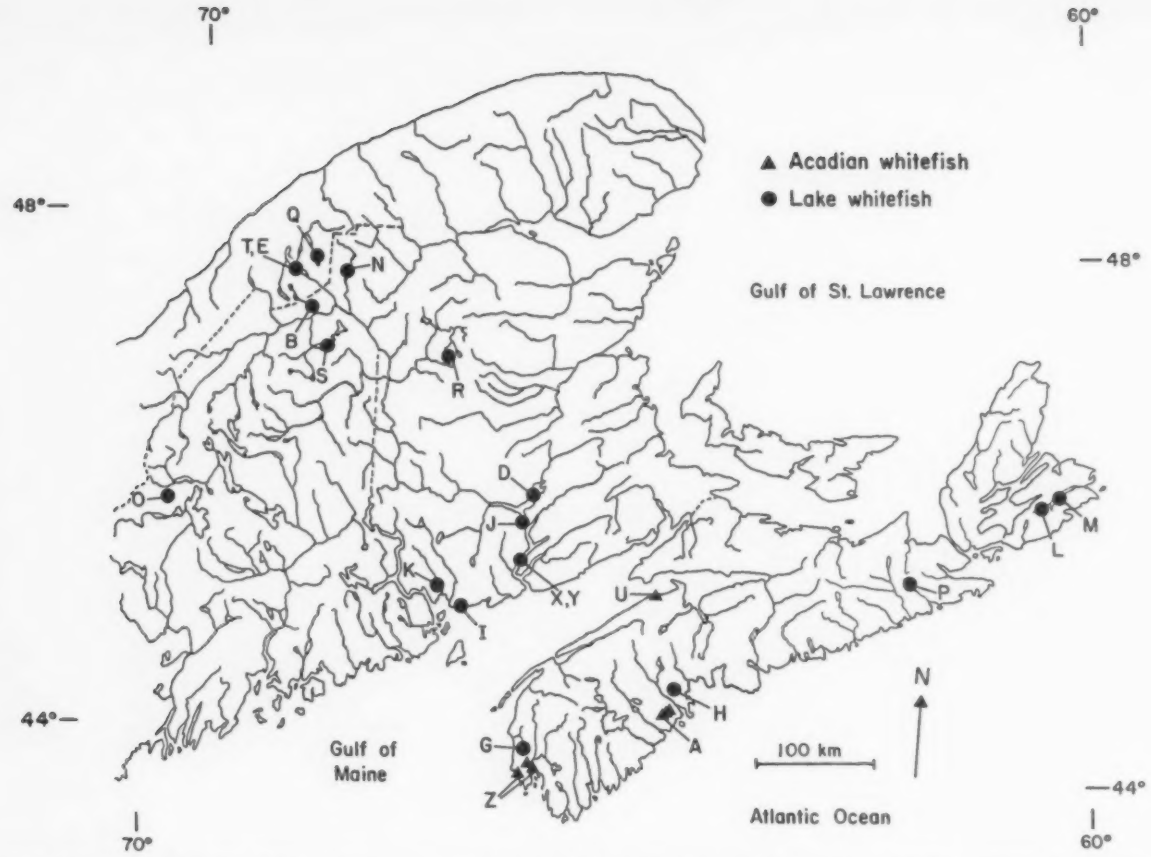

FIG. 1. Geographic distribution of whitefish collections examined from the Maritimes region. Acadian whitefish (▲): A, Minamkeak, Milipsigate, and Hebb lakes; U, Hall's Harbour; Z, Tusket River, Annis River, Yarmouth Harbour. Lake whitefish (●): B, Baker Lake, D, Grand Lake; E, Lake Temiscouata (1980); G, Lake George; H, Little Mushamush and Caribou lakes; I, Black's Harbour; J, Saint John River; K, Kerr Lake; L, Salmon River; M, Mira River; N, Third Lake; O, Long Pond; P, Pringle Lake; Q, Squatook Lake; R, Trousers Lake; S, Square Lake; T, Lake Temiscouata (1951); X and Y, Saint John River.

complex, *Coregonus clupeaformis*, is not as apparent. The lake whitefish is the only other whitefish species found in Nova Scotia and these two species have been confused by Piers (1927), Dymond (1947), Smith (1952), Bigelow and Schroeder (1953), Livingstone (1953), Leim and Day (1959), Bigelow (1963), and Semple (1973). The lake whitefish has been shown to exhibit a wide range of phenotypic and genotypic variation across North America (Koelz 1929, 1931; Fenderson 1964; Lindsey et al. 1970; Franzin and Clayton 1977; Ihssen et al. 1981b). Sympatric lake whitefish species pairs are also widely known (Kennedy 1943; Lindsey 1963; Fenderson 1964; Bodaly 1979; Kirkpatrick and Selander 1979; Lindsey 1979; Bruce 1984; Bodaly et al. 1988; Bernatchez and Dodson 1990). This wide range of morphological and ecological variation has presented a problem of distinguishing the Acadian whitefish from the lake whitefish species complex; indeed some doubt has been expressed that the Acadian whitefish was a distinct species.

Scott and Crossman (1973) distinguished the Acadian whitefish on the basis of having more lateral line scales (91–100) than lake whitefish (70–85) and by the presence of a terminal mouth (or nearly so) with small but well-developed teeth in the adults. The lake whitefish was reported to have an obviously subterminal mouth lacking such teeth except in juveniles under 100 mm. However, lake whitefish have been reported to have lateral line scale counts as high as 97 (Koelz 1931; Scott and Crossman 1973) and individual variation in mouth shape or in the location and development of teeth has not been well characterized in the two species. There has never been a taxonomic study of the Acadian whitefish and the following study was conducted to test the morphological basis for recognizing the Acadian whitefish and lake whitefish as distinct species. Multivariate analyses of 54 morphological characters examined the degree to which Acadian whitefish were distinguished from the lake whitefish species complex. In addition, diagnostic morphological characters were sought for the Acadian whitefish.

Materials and Methods

Whitefish specimens examined in this study were obtained from museums or were collected during field surveys conducted in Nova Scotia between 1982 and 1985. Additional data on whitefish collections examined in this study are presented in Table 1 (NMC, Canadian Museum of Nature, Ottawa, Ont.; ROM, Royal Ontario Museum, Toronto, Ont.; USNM, United

States National Museum, Washington, DC). The geographic distribution of these collections in the Canadian Martimes region is shown in Fig. 1. All morphological data collected in this study are deposited in the Canadian Museum of Nature (formerly the National Museum of Natural Sciences), Ottawa, Ont. (NMC).

A total of 49 Acadian whitefish and 211 lake whitefish specimens were examined in the study of morphological variation. Ten meristic characters were counted and 44 morphometric characters were measured. Because some of the Acadian whitefish and lake whitefish specimens were damaged, not all characters could be recorded for every specimen. Meristic counts were made on the left side of specimens under a dissecting microscope although counts on larger specimens were made by eye. Vertebrae counts were made from radiographs and did not include the urostyle. Morphometric measurements were made on the left side of specimens with digital Yushio calipers to 0.01 mm accuracy. The characters examined included the following: snout to anal length, dorsal to adipose length, pectoral to ventral length, ventral to anal length, adipose to caudal length, and eyeball diameter following Koelz (1931); dorsal, anal, pectoral, and pelvic fin rays, branchiostegal rays, lateral

FIG. 2. Acadian whitefish (top) with terminal mouth and lake whitefish (bottom) with subterminal mouth and pearl organs. Acadian whitefish was caught November 12, 1982, in Hebb Lake, N.S. (NMC 82–806, specimen Y3904); SL = 230.7 mm. Lake whitefish was caught November 16, 1958, in Grand Lake, N.B. (ROM 21677, specimen 4); SL = 226.1 mm.

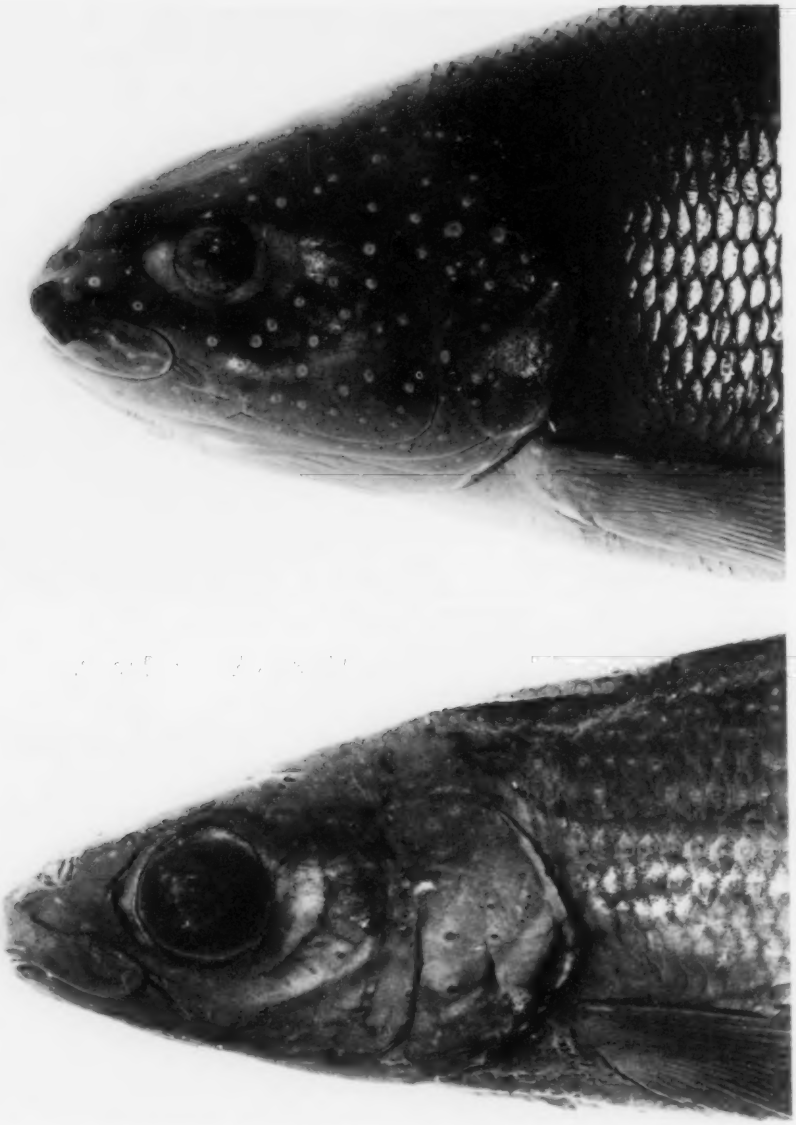

FIG. 3. Acadian whitefish (top) with slightly overhanging upper jaw and pearl organs and lake whitefish (bottom) with nearly terminal mouth. Acadian whitefish was caught November 1954 in Tusket River, N.S. (ROM 16674); SL = 475.4 mm. Lake whitefish was caught November 10, 1903, in Square Lake Thoroughfare, ME (USNM 88414, specimen 20); SL = 185.6 mm.

line scales, scales above the lateral line, scales below the lateral line, gill rakers, vertebrae, width of gape, postorbital length, upper jaw length, height of dorsal and anal fins, length of pectoral and pelvic fins, length of dorsal and anal fin bases, predorsal length, caudal peduncle depth, snout length and standard length following Hubbs and Lagler (1958); interorbital width, maxillary width and adipose fin length following Lindsey (1962); predorsal depth and anal depth following Loch (1974); eye diameter following Bodaly (1979); and head length, orbital diameter, head depth, and gill raker length following Casselman et al. (1981). Additional characters examined are defined as follows: snout to eye margin length is the distance between the anterior tip of the snout and the posterior margin of the skin in front of the eye; suborbital depth is the vertical distance between the ventral margin of the orbit and the ventral contour of the head; pupil diameter is the horizontal diameter of the pupil; nare diameter is the width of the anterior nare flap at its base; internaral width is the distance between the medial bases of the anterior nare flaps; pectoral and pelvic fin base lengths are measured by drawing the fin away from the body and lightly clamping the fin base from ventral side as close as possible to the body with calipers; prepectoral length is the distance from the tip of the snout to the base of the first anterior pectoral fin ray; prepelvic length is the distance from the tip of the snout to the base of the first anterior pectoral fin ray; anal to caudal length is the distance from the origin of the anal fin to the base

TABLE 2. Variation of meristic characters between whitefish populations showing mean (standard erro
lateral line scales; DFR, dorsal fin rays; AFR, anal fin rays; PCFR, pectoral fin rays; PLFR, pelvic f
frequency.

Locality	SAL	SBL	LLS	DFR	AFR
					Acadi
Hebb Lake, N.S.	10.30 (0.483) 10	9.00 (0.0) 8	95.00 (2.507) 8	13.58 (0.669) 12	14.50 (0.756) 8
Milipsigate L., N.S.	10.88 (0.641) 8	8.89 (0.333) 9	92.00 (2.887) 7	13.67 (0.778) 12	14.10 (0.568) 10
Minamkeak L., N.S.	10.75 (0.500) 4	9.00 (0.000) 3	91.00 (4.243) 2	13.80 (0.837) 5	14.20 (0.447) 5
Annis River, N.S.	10.00 (0.000) 2	8.00 (0.000) 2	95.50 (0.707) 2	15.00 (0.000) 2	15.00 (0.000) 2
Tusket River, N.S.	10.85 (0.376) 13	9.15 (0.376) 13	94.23 (3.468) 13	14.69 (0.480) 13	13.92 (0.760) 13
Total/mean	10.65 (0.538) 37	9.03 (0.296) 35	93.81 (3.187) 32	14.01 (0.821) 44	14.18 (0.692) 38
					Lake
Mushamush R. Lakes	9.67 (0.577) 3	9.00 (0.000) 2	78.33 (5.033) 3	13.67 (0.577) 3	16.00 (0.000) 3
Lake George, N.S.	10.00 (0.471) 19	8.63 (0.496) 19	81.20 (3.075) 15	14.16 (0.765) 19	16.32 (0.749) 19
Pringle Lake, N.S.	10.57 (0.535) 7	9.71 (0.488) 7	75.00 (2.236) 7	14.29 (0.756) 7	14.86 (0.378) 7
Salmon River, N.S.	10.40 (0.548) 5	9.40 (0.548) 5	68.00 (1.414) 5	13.60 (0.548) 5	14.20 (0.447) 5
Mira River, N.S.	9.51 (0.689) 37	8.31 (0.467) 36	70.06 (2.904) 33	13.97 (0.788) 38	14.86 (0.762) 36
Grand Lake, N.B.	9.67 (0.492) 12	8.55 (0.522) 11	76.67 (3.892) 12	14.00 (0.426) 12	15.25 (0.622) 12
St. John River, N.B.	10.10 (0.447) 20	8.10 (0.308) 20	77.10 (2.789) 20	13.95 (0.686) 20	14.60 (0.821) 20
Kerr Lake, N.B.	10.61 (0.722) 23	8.57 (0.598) 21	69.32 (3.061) 22	13.91 (0.996) 23	14.48 (0.730) 23
Square Lake, ME	9.87 (0.344) 23	8.17 (0.388) 23	78.39 (3.526) 23	13.61 (0.583) 23	15.83 (0.650) 23
L. Temiscouata, Que.	10.03 (0.400) 32	8.61 (0.495) 31	85.69 (3.393) 32	13.41 (0.762) 32	15.97 (0.933) 32
Long Pond, ME	10.11 (0.333) 9	8.35 (0.452) 12	74.08 (2.314) 12	14.17 (0.577) 12	16.33 (0.888) 12
Baker Lake, N.B.	10.67 (0.577) 3	9.00 (1.000) 3	79.33 (2.887) 3	13.33 (0.577) 3	15.33 (0.577) 3
Trouser's L., N.B.	10.33 (0.471) 3	8.67 (0.577) 3	82.33 (2.887) 3	14.33 (0.577) 3	15.00 (0.000) 3
Third Lake, N.B.	11.00 (0.000) 2	9.50 (0.707) 2	81.50 (3.536) 2	13.00 (0.000) 2	15.50 (0.707) 2
Total/mean	10.02 (0.634) 205	8.50 (0.593) 201	76.64 (6.434) 198	13.85 (0.767) 209	15.33 (0.993) 208

rd error) and sample size. SAL, scales above lateral line; SBL, scales below lateral line; LLS, pelvic fin rays; BRR, branchiostegal rays; GR, gill rakers, VERT, vertebrae; ENF, eyelid notch

FR	PCFR	PLFR	BRR	GR	VERT	ENF
Acadian whitefish						
(0.756)	16.64 (0.505)	12.00 (0.408)	8.80 (0.422)	26.64 (0.809)	65.70 (0.823)	
8	11	13	10	11	10	0.70
(0.568)	16.55 (0.522)	12.07 (0.267)	8.67 (0.651)	26.18 (0.951)	64.86 (0.690)	
0	11	14	12	11	7	0.90
(0.447)	17.00 (0.000)	12.00 (0.000)	8.40 (0.894)	27.80 (1.633)	65.00 (1.000)	
5	5	5	5	4	3	0.60
(0.000)	16.50 (0.707)	12.00 (0.000)	8.50 (0.707)	26.50 (0.707)	66.00 (0.000)	
2	2	2	2	2	2	1.00
(0.760)	16.92 (0.494)	12.23 (0.439)	8.46 (0.529)	26.46 (0.776)	65.08 (0.494)	
13	13	13	13	13	13	0.91
(0.692)	16.74 (0.497)	12.09 (0.351)	8.60 (0.587)	26.49 (0.870)	65.26 (0.741)	
88	42	47	42	41	35	0.82
Lake Whitefish						
(0.000)	18.67 (0.577)	12.33 (0.577)	9.00 (1.000)	26.33 (1.155)	60.00 (1.000)	
3	3	3	3	3	3	0.00
(0.749)	17.33 (0.730)	12.00 (0.324)	8.71 (0.717)	25.14 (0.964)	61.33 (0.840)	
19	21	20	21	21	18	0.00
(0.378)	18.14 (0.690)	12.43 (0.535)	8.71 (0.488)	30.86 (0.690)	59.43 (0.787)	
7	7	7	7	7	7	0.00
(0.447)	17.00 (0.707)	12.20 (0.447)	9.20 (0.447)	23.20 (0.837)	60.00 (0.707)	
5	5	5	5	5	5	0.50
(0.762)	16.55 (0.828)	11.97 (0.677)	8.92 (0.673)	22.58 (0.894)	60.47 (0.615)	
36	38	38	38	36	34	0.00
(0.622)	17.33 (0.651)	12.50 (0.522)	8.92 (0.515)	24.33 (1.155)	59.67 (0.651)	
12	12	12	12	12	12	0.25
(0.821)	16.40 (0.598)	12.10 (0.308)	8.70 (0.470)	24.50 (1.192)	60.35 (1.226)	
20	20	20	20	20	20	0.00
(0.730)	17.70 (0.559)	12.30 (0.470)	8.70 (0.559)	30.14 (0.990)	59.35 (0.647)	
23	23	23	23	22	23	0.43
(0.650)	16.43 (0.788)	12.26 (0.449)	8.22 (0.422)	25.00 (1.446)	61.29 (0.722)	
23	23	23	23	23	23	0.04
(0.933)	16.50 (0.762)	12.09 (0.296)	8.78 (0.659)	25.41 (1.316)	61.31 (1.061)	
32	32	32	32	32	32	0.00
(0.888)	16.17 (0.577)	12.75 (0.452)	8.75 (0.452)	27.83 (0.937)	60.67 (1.073)	
12	12	12	12	12	12	0.17
(0.577)	17.00 (1.000)	12.00 (0.000)	8.33 (0.577)	26.33 (0.577)	61.33 (0.577)	
3	3	3	3	3	3	0.33
(0.000)	16.00 (1.000)	12.00 (0.000)	8.33 (0.577)	23.33 (0.577)	62.00 (1.000)	
3	3	3	3	3	3	0.00
(0.707)	16.00 (0.000)	12.50 (0.707)	8.50 (0.707)	27.50 (2.121)	62.50 (0.707)	
2	2	2	2	2	2	1.00
(0.993)	16.84 (0.993)	12.19 (0.500)	8.73 (0.622)	25.44 (2.754)	60.61 (1.138)	
208	211	210	211	204	202	0.11

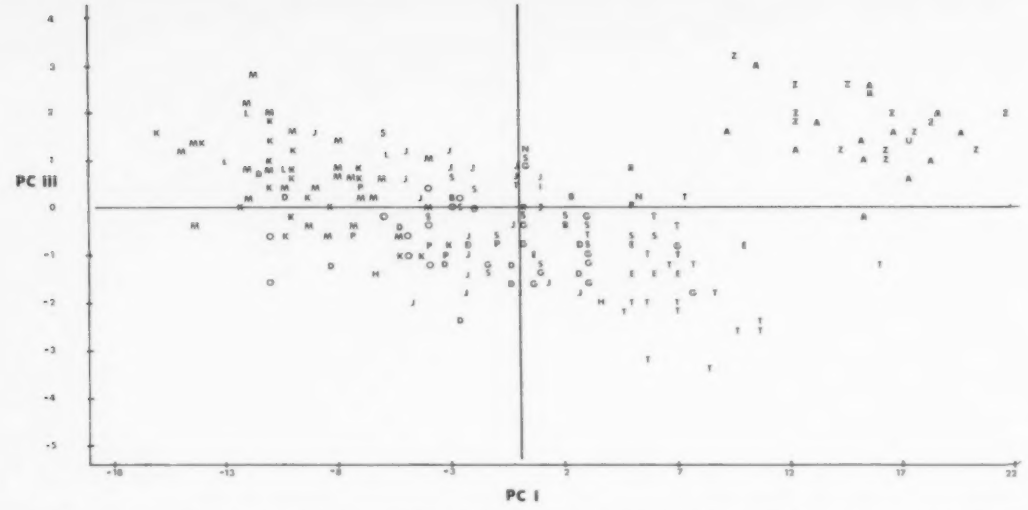

FIG. 4. Principal component analysis summarizing covariation of 10 meristic characters. Acadian whitefish specimens are coded by A (Petite Rivière lakes), U (Hall's Harbour), and Z (Tusket River watershed). For lake whitefish specimen codes, see Fig. 1.

of the first lower procurrent caudal ray; pelvic axillary process length is the distance from the base of the first anterior pelvic ray horizontally back to the posterior free tip of the pelvic axillary scale; prepectoral depth is the depth of the body through the origin of the pectoral fin; isthmus length is the distance from the anterior tip of the lower jaw to the notch where the branchiostegal membranes join; adipose base length is the distance from the origin of the adipose fin to the posterior base of the fin as grasped lightly by calipers; gill filament length is the length of the longest gill filament at the sharpest angle of the first left gill arch.

It has been suggested that in morphometric studies, fish populations should be compared in terms of shape variates free from the effects of size variation (Reist 1985, 1986). In this study, size effects were minimized in the morphometric data using the regression-related technique of calculating residuals. All morphometric data were first transformed to natural logarithms and standardized to mean = 0 and standard deviation = 1. The morphometric characters were then transformed into shape variates (residuals) by expressing them as the deviation of individuals from the pooled within-group regression line describing the size relationship between the character and standard length. This deviation, orthogonal to the size relationship, should be independent of size and reflect the residual variation resulting from measurement error and the biological deviation of individuals from the structural relationship (Kuhry and Marcus 1977). These residuals were then used as the morphometric characters in subsequent univariate and multivariate analyses.

The covariation of characters was investigated by principal component analysis (PCA) using the Statistical Analysis System (SAS) Princomp procedure. Meristic and morphometric characters were analyzed separately as suggested by Ihssen et al. (1981a). The analysis used a variance/covariance data matrix. Meristic and morphometric characters were also analyzed together using a correlation coefficient matrix, since the variation between characters measured in different units could be a function of scaling (Neff and Marcus 1980). After identification of Acadian whitefish and lake whitefish using PCA, a canonical variate analysis (CVA) using the SAS Canvar procedure was performed to test for a statistical difference between the two taxa. An ANOVA and a stepwise discriminant analysis accounting for character correlation were also performed to test which characters were significantly different between the Acadian whitefish and the lake whitefish.

Results

One of the characters used by Scott and Crossman (1973) to distinguish Acadian whitefish and lake whitefish was mouth shape. Examination of whitefish specimens in this study confirmed mouth shape to be a good external diagnostic character distinguishing these two taxa. The Acadian whitefish had a rounder snout profile and a terminal mouth rather than the more pointed snout profile and subterminal mouth characteristic of lake whitefish (Fig. 2). However, individual variation in mouth shape was found in both taxa, with a few Acadian whitefish having a slightly overhanging upper jaw and a few lake whitefish having a nearly terminal mouth (Fig. 3). The reliability of this character to distinguish Acadian whitefish is very high although it will vary depending on the experience of the observer. Specimens in Fig. 2 and 3 also show the different type and arrangement of pearl organs on the heads of Acadian and lake whitefish approaching spawning condition. The pearl organs on the heads of Acadian whitefish were generally larger, less numerous, and had a more regular circular shape than those of lake whitefish. Lake whitefish were also observed to have prominent rows of ridged pearl organs on scales along the flanks of specimens. Such pearl organs were not present on any Acadian whitefish. As well, a ventral notch in the adipose eyelid was observed on many Acadian whitefish. This notch was found on 31 Acadian whitefish (82% of the specimens examined) compared with only 19 lake whitefish (11% of the specimens examined).

A summary of meristic data is presented in Table 2. From the study of additional specimens unavailable to Scott and

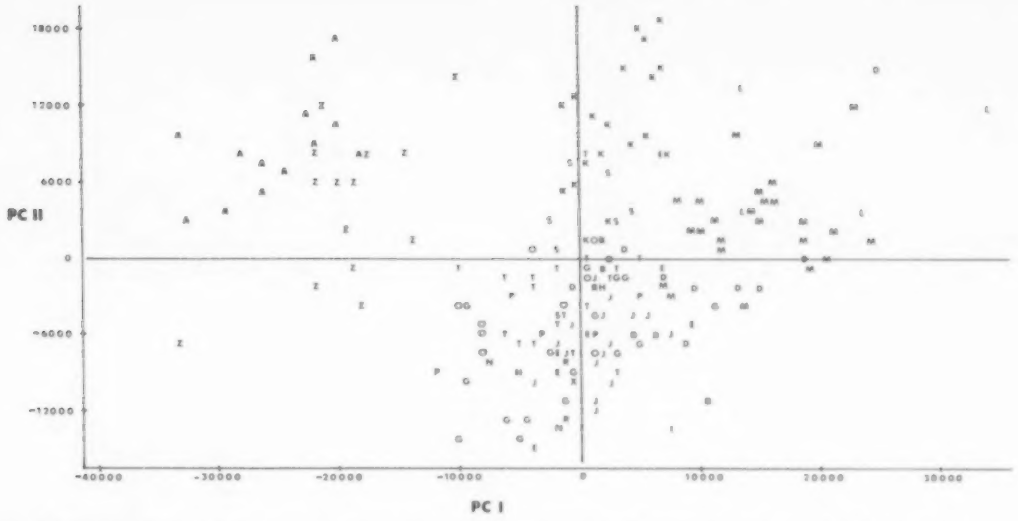

FIG. 5. Principal component analysis summarizing covariation of 43 morphometric characters. Acadian whitefish specimens are coded by A (Petite Rivière lakes) and Z (Tusket River watershed). For lake whitefish specimen codes, see Fig. 1.

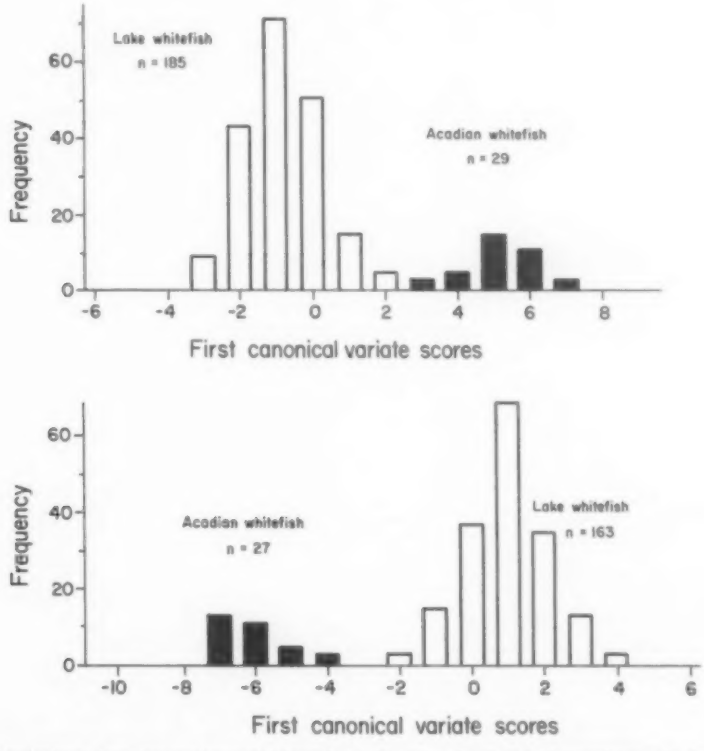

FIG. 6. Discriminant function analyses of 10 meristic characters (top) and 43 morphometric characters (bottom).

Crossman (1973) and Scott and Scott (1988), the ranges of several meristic characters for the Acadian whitefish were extended. The PCA of this meristic data distinguished all Acadian whitefish specimens from lake whitefish specimens (Fig. 4). The first principal component (PC I) accounted for 86% of the variation in the data and weighted lateral lines scales most heavily followed by vertebrae and gill rakers. PC III accounted for 2% of the variation and vertebrae count was weighted most heavily followed by anal fin rays and lateral line scales. A plot of PC I against PC II indicated good although

TABLE 3. Morphological characters showing a significant difference between Acadian whitefish and lake whitefish ($p < 0.01$). Meristic characters are raw data, while morphometric characters are residual values.

Character	Acadian whitefish No.	Acadian whitefish $\bar{X}$	Lake whitefish No.	Lake whitefish $\bar{X}$	F value	Prob $>F$
Vertebrae	35	65.26	202	60.61	543.40	0.0001
Adipose–caudal length	33	−7190.33	204	884.66	310.00	0.0001
Adipose base length	33	−11174.55	204	1566.25	278.94	0.0001
Nare diameter	33	−11589.64	203	1623.34	221.02	0.0001
Lateral line scales	32	93.81	198	76.64	218.63	0.0001
Pelvic axillary process length	33	7214.58	204	−1180.51	207.53	0.0001
Adipose fin length	33	−7755.67	202	895.32	172.04	0.0001
Height of anal fin	31	−4962.39	186	682.62	162.07	0.0001
Eye diameter	33	−7248.42	200	1350.50	158.47	0.0001
Pectoral fin length	33	−4542.88	202	749.79	125.71	0.0001
Pectoral–ventral length	33	2635.15	204	−430.74	97.64	0.0001
Predorsal length	33	1538.67	203	−171.69	92.54	0.0001
Prepelvic length	33	1304.61	203	−208.00	84.96	0.0001
Anal fin rays	38	14.18	208	15.33	50.84	0.0001
Anal base length	32	−2903.22	204	358.87	45.88	0.0001
Anal–caudal length	32	−2363.47	204	320.79	42.54	0.0001
Ventral–anal length	32	−1764.69	204	230.03	37.01	0.0001
Width of gape	33	2353.30	203	−678.38	36.76	0.0001
Maxillary width	33	−2884.97	203	533.07	36.14	0.0001
Scales above lateral line	37	10.65	205	10.02	32.24	0.0001
Upper jaw length	33	1823.97	203	−344.77	28.67	0.0001
Scales below lateral line	35	9.03	201	8.50	26.37	0.0001
Pectoral base length	33	−2119.27	204	220.93	25.80	0.0001
Anal depth	32	−1186.00	204	224.28	22.41	0.0001
Dorsal base length	33	−1504.94	204	277.51	20.67	0.0001
Gill filament length	32	1344.60	196	−240.46	14.30	0.0002
Eyeball diameter	33	−1985.36	199	436.64	13.71	0.0003
Pupil diameter	33	−2768.70	200	978.12	12.74	0.0004
Interorbital width	32	1131.81	201	−70.00	12.42	0.0005
Preanal length	32	343.13	203	−62.18	10.46	0.0014
Prepectoral length	33	−853.70	203	207.82	9.68	0.0021
Prepectoral depth	31	−755.23	204	142.82	9.14	0.0028
Internaral width	32	1732.09	203	−122.32	8.39	0.0041
Isthmus length	31	1444.45	199	−285.66	7.54	0.0065
Predorsal length	33	−732.15	204	114.46	7.01	0.0086

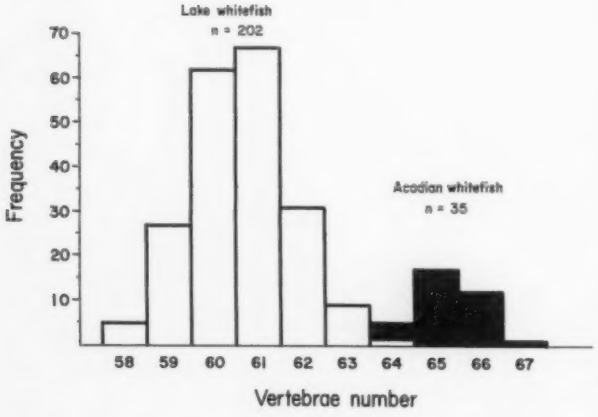

FIG. 7. Frequency histogram of vertebrae number.

not complete separation of Acadian whitefish from lake whitefish. Since all biologically important variation is not necessarily found on the first two axes of a PCA (Neff and Marcus 1980) and because the data were being analyzed for group structure, the existence of two clusters separated by a distinct gap in Fig. 4 was interpreted as indicating that all the Acadian whitefish could be distinguished from lake whitefish specimens based on meristic characters.

When PCA was performed on morphometric data, two distinct clusters corresponding to Acadian whitefish and lake whitefish were also discernible (Fig. 5). PC I accounted for 33% of the data variation and weighted nare diameter most heavily followed by adipose fin base length and adipose fin length. PC II accounted for 14% of the variation and gill raker length was weighted most heavily followed by isthmus length and length of the pelvic axillary process. Acadian whitefish and lake whitefish also formed two distinct clusters when the morphometric data were transformed to logarithms rather than residuals and size effects were minimized by removing PC I. The subsequent plot of PC II against PC III (shape components) accounted for 3% of the variation in the data whereas PC I (size

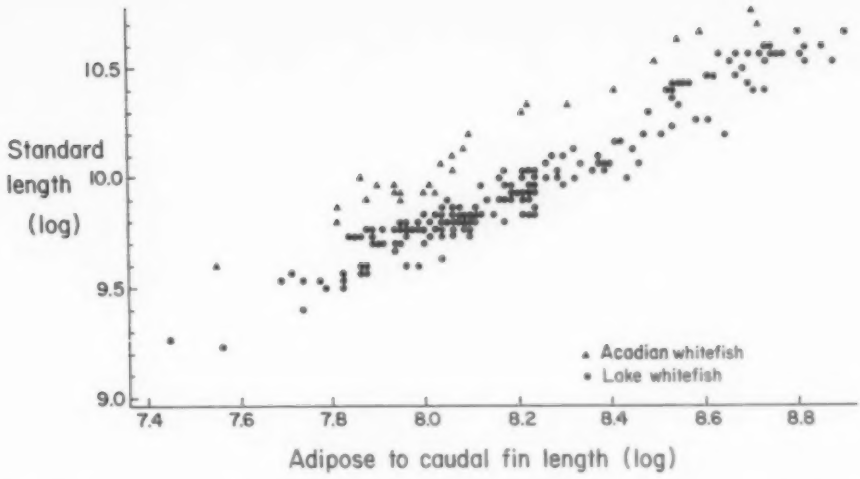

FIG. 8. Adipose to caudal fin length (log) distinguishing Acadian whitefish and lake whitefish specimens.

component) accounted for 92% of the variation. Acadian whitefish and lake whitefish were also clearly distinguished when the meristic and morphometric data were analyzed together by PCA using the correlation coefficient matrix.

Discriminant function analyses (canonical variate axis I) of meristic and morphometric data sets clearly distinguished (significant at $p < 0.0001$) all Acadian whitefish from lake whitefish (Fig. 6). In the analysis of meristic data, the variables giving the most separation between these two taxa were vertebrae, anal fin rays, and scales above the lateral line. The variables giving the most separation in the analysis of morphometric data were prepectoral depth, predorsal length, and pectoral fin length.

An ANOVA on meristic and morphometric characters showed that the Acadian whitefish and lake whitefish were significantly different ($p < 0.01$) for 35 characters (Table 3). The best of these taxonomic characters for distinguishing Acadian whitefish from lake whitefish was the number of vertebrae. This diagnostic character could correctly identify over 97% of the specimens examined (Fig. 7). Only one lake whitefish specimen from Lake Temiscouata, Quebec, was found to have a vertebrae number (64) that overlapped the range found for Acadian whitefish (64–67). This specimen also had a lateral line scale number (90) that overlapped with Acadian whitefish (88–100) but it was classified as a lake whitefish in multivariate analyses of morphological variation and by its subterminal mouth. The best distinguishing morphometric character showed the Acadian whitefish to have a shorter adipose fin to caudal fin length than lake whitefish (Fig. 8). The number of lateral line scales was also a useful taxonomic character to distinguish Acadian whitefish (88–100, $\bar{X} = 93.8$) from lake whitefish (63–95, $\bar{X} = 76.6$). Gill raker number was not significantly different between Acadian whitefish (25–29, $\bar{X} = 26.5$) and lake whitefish (20–32, $\bar{X} = 25.4$).

An ANOVA showed anadromous Acadian whitefish from the Tusket River watershed to be significantly different ($p < 0.01$) from landlocked Acadian whitefish in the Petite Riviere watershed lakes for 19 characters. These characters in order of most significant difference were gill raker length, number of dorsal fin rays, length of pelvic fin, pectoral fin base length, height of anal fin, caudal peduncle depth, height of dorsal fin, length of pectoral fin, pelvic fin base length, adipose fin length, internaral width, pectoral–ventral length, maxillary width, prepelvic length, snout length, orbital diameter, nare diameter, upper jaw length, and anal to caudal length.

The lake whitefish species complex was found to show considerable morphological variation between populations in the Maritimes region. Lateral line scale counts from the Mira River, N.S. (64–75, $\bar{X} = 70.1$), and Kerr Lake, N.B. (63–75, $\bar{X} = 69.3$), were unusually low for the species. Gill raker counts were also variable. High counts were observed in populations from Kerr Lake (28–32, $\bar{X} = 30.1$) and Pringle Lake, N.S. (30–32, $\bar{X} = 30.9$), while the Mira River population (20–24, $\bar{X} = 22.6$) had unusually low gill raker counts for eastern North America. Specimens of whitefish from Square Lake, Maine, and Lake Temiscouata showed considerable similarity in head shape having a more rounded snout profile and a mouth that could often be described as nearly terminal. This unique head shape (see Fig. 3) was one of the characters used to describe a new species (*Coregonus stanleyi*) from the Cross Lake Thoroughfare, Maine, by Kendall (1903). However, the present multivariate analyses did not find sufficient morphological differences to recognize whitefish specimens from Square Lake (identified as *C. stanleyi*) or Lake Temiscouata as taxonomically distinct from the other populations of lake whitefish examined.

Discussion

The univariate and multivariate analyses of morphological data in this study indicated that there were highly significant phenotypic differences between Acadian whitefish and lake whitefish. Acadian whitefish and lake whitefish were significantly different for 35 morphological characters examined and the PCA of meristic and morphometric variation showed complete separation of Acadian whitefish from lake whitefish. This degree of morphological separation in PCA has been implied as a suitable criterion for distinguishing species (Smith 1973; Neff and Marcus 1980; Todd et al. 1981). The morphological evidence for recognizing the Acadian whitefish as a species distinct from the lake whitefish is supported by a recent study of isozymes and mitochondrial DNA which found significant

genetic differences between Acadian whitefish and lake whitefish also indicating that they were distinct species (Bernatchez et al. 1991).

The Acadian whitefish could be reliably distinguished from the lake whitefish based on several morphological characters. The best diagnostic character for the Acadian whitefish was found to be vertebral number. While this character is known to be influenced by environmental factors (Tåning 1952; Fowler 1970; Lindsey and Arnason 1981), the difference between Acadian whitefish (64–67, $\bar{X}$ = 65.3) and lake whitefish (58–64, $\bar{X}$ = 60.6) probably reflects genetic differences considering that the range of vertebrae counts for lake whitefish in different climatic zones across North America is consistently lower at 55–64 (Scott and Crossman 1973). The Acadian whitefish also showed a different type and arrangement of pearl organs than lake whitefish. Pearl organs are epidermal structures that are thought to function primarily in facilitating contact between individuals during spawning (Vladykov 1970). Since they are closely associated with reproduction, developing only during spawning season, the different pearl organs exhibited by Acadian whitefish and lake whitefish are likely species specific. The terminal mouth of the Acadian whitefish was notably different from the subterminal mouth of the lake whitefish. The different mouth shapes of these species could be related to adaptive differences in behavioral traits such as feeding habits (Edge 1987). The present study also found that most Acadian whitefish (82%) had a ventral notch in the adipose eyelid compared with only 11% of the lake whitefish examined. This notch was reported by McPhail and Lindsey (1970) to be present in ciscos (subgenus *Leucichthys*) and whitefish species of the genus *Prosopium* but absent in the whitefishes (subgenus *Coregonus*).

While it was possible to readily distinguish the Acadian whitefish from the morphological variation found within the lake whitefish species complex, no morphological basis was found for recognizing any lake whitefish populations from the Maritimes region as taxonomically distinct. However, lake whitefish showed considerable morphological variation in the Maritimes region, with several populations having clearly nonoverlapping counts of gill rakers. Gill raker number has long been used to study lake whitefish population structure because of its strong genetic basis (Lindsey et al. 1970; Loch 1974; Ihssen et al. 1981a, 1981b) and although not immune to environmental modification (Lindsey 1981), these gill raker differences probably reflect genetic differences between populations.

It had been anticipated that whitefish specimens examined from Square Lake might have shown a morphological distinctiveness that reflected their prior taxonomic recognition. The specimens from Square Lake had been caught in 1903 by W. C. Kendall and were identified as *Coregonus stanleyi*. Stanley's whitefish, *C. stanleyi*, was described by Kendall (1903) from the Cross Lake Thoroughfare, just upstream from Square Lake, and it was distinguished from the lake whtiefish, its most similar relative, mainly on the basis of a more "sharply rounded" and less truncated snout. Stanley's whitefish was also characterized by a "shorter appearance of the head" and, on average, more and longer gill rakers than the lake whitefish. While morphological evidence for recognizing Stanley's whitefish was not strong, Kendall also reported its smaller adult size (dwarf) and earlier spawning period which did not overlap with the period for lake whitefish in the same lake. There has not been a proper taxonomic study of *C. stanleyi* since its first description. While studies by Fenderson (1964), Kirkpatrick and Selander (1979), and Bernatchez and Dodson (1990) have provided evidence to support its ecological and genetic distinctiveness, this species (sensu Mayr 1969) remains difficult to distinguish morphologically. Further investigation will be required to determine whether *C. stanleyi* warrants taxonomic recognition.

The morphological data in this study indicate that the Acadian whitefish shows affinities to both the *Coregonus* and *Leucichthys* subgenera. On the basis of gill raker counts, traditionally a diagnostic character in whitefish taxonomy (Svardson 1952; Lindsey 1981), the Acadian whitefish would be classified in the subgenus *Coregonus* (whitefishes) and would appear most closely related to the lake whitefish. However, the Acadian whitefish shows similarities to the subgenus *Leucichthys* (ciscos) based on its terminal mouth and adipose eyelid notch. Behnke (1972) also observed that the shape of the Acadian whitefish maxilla was more similar to that of the ciscos (subgenus *Leucichthys*) than of whitefishes (subgenus *Coregonus*). On the assumption that both *Coregonus* and *Leucichthys* subgenera are monophyletic, the Acadian whitefish could represent a specialized species that recently evolved in either subgenus, or an evolutionary lineage that had its origin around the time of divergence of whitefishes and ciscos. Genetic studies by Bernatchez et al. (1991) suggested that the Acadian whitefish evolved well before the Wisconsinian glaciation period and that the species did not represent a recently specialized species of either subgenera. Further comparative studies of morphological, osteological, and genetic characters and their phylogenetic polarity in other coregonine species will be required to properly understand the systematics of the Acadian whitefish. These studies must be conducted soon, as the habitat of the Acadian whitefish is becoming increasingly acidified (Watt et al. 1983; Edge 1987). The extinction of this species would represent a serious loss of genetic diversity and potential knowledge of coregonid evolution and zoogeography.

Acknowledgements

The authors would like to thank W. B. Scott, E. J. Crossman, J. Gilhen, R. Dittman, D. Percival, and L. Earl for assistance in obtaining whitefish specimens. J. McNeil provided much assistance with statistical analyses, Mr. Ben-Tchavtchavdze prepared the photographs, J. Hélie prepared the figures, and Sylvie Lacombe assisted with preparation of the manuscript.

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Genetic Variation and Fitness: A Test in a Naturalized Population of Brook Trout (*Salvelinus fontinalis*)

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The relationship between enzyme heterozygosity and several components of fitness (size and survival) was examined in a naturalized population of brook trout (*Salvelinus fontinalis*) from Algonquin Provincial Park, Ontario. Significant differences in allele frequency and the number of heterozygous loci per individual were found among five age classes spanning most of the life history stages of brook trout in this population. A young of the year (YOY) cohort sampled after a period of high natural mortality had significantly greater numbers of heterozygous loci per individual than YOY sampled shortly after emergence from redd sites. A positive association between heterozygosity and size was only found in YOY sampled in June and not in the other age classes in which sexual maturity was not a complicating factor. The association between heterozygosity and size was affected by the onset of sexual maturity. Significant negative regressions between multilocus or single locus heterozygosity and size were found in mature males whereas heterozygosity was positively associated with size in females. These dissimilarities may be the result of differences in growth rates prior to sexual maturation and differential allocation of energy towards gametic and somatic tissue.

On a étudié la relation entre l'hétérogénie des enzymes et plusieurs composantes de la condition physique (taille et taux de survie) dans une population acclimatée d'omble de fontaine (*Salvelinus fontinalis*) du parc provincial Algonquin (Ontario). On a relevé des différences significatives de la fréquence allélique et du nombre de loci hétérozygotes par poisson chez cinq classes d'âges englobant la presque totalité du cycle vital de l'omble de fontaine composant cette population. L'échantillonnage d'une cohorte d'ombles de moins d'un an après une période de mortalité naturelle élevée a révélé un nombre nettement plus élevé de loci hétérozygotes par poisson comparativement aux ombles du même âge échantillonnés peu après avoir quitté les sillons. Une association positive entre l'hétérogénie et la taille n'a été relevée que chez les ombles de moins d'un an échantillonnés en juin et non chez les autres classes d'âges où la maturité sexuelle n'était pas un facteur complexant. L'approche de la maturité sexuelle influait sur l'association entre l'hétérogénie et la taille. On a aussi relevé des régressions négatives significatives entre, d'une part, l'hétérogénie à plusieurs loci et l'hétérogénie à un seul locus et, d'autre part, la taille des mâles matures, tandis que l'hétérogénie montrait une association positive avec la taille des femelles. Ces différences peuvent être le résultat de variations du taux de croissance avant la maturité sexuelle et une répartition différentielle de l'énergie pour l'élaboration de tissus somatiques et de gamètes.

Received September 7, 1990
Accepted May 1, 1991
(JA716)

Reçu le 7 septembre 1990
Accepté le 1 mai 1991

The number of hatchery programs that produce fish for release into natural bodies of water is continually increasing. Producing and maintaining a biological resource that will live and reproduce in the wild is an important management goal. Preserving genetic variation (heterozygosity) in hatchery stocks is recognized as playing a vital role in this regard. The loss of genetic variation is expected to reduce the ability of populations to make adaptive responses to altered environmental conditions (Allendorf and Leary 1988). Poorer survival and growth, atypical morphology, and abnormal development are just a few of the negative effects documented in hatchery populations of fish suffering from a loss of genetic variation (reviewed by Kirpichnikov 1981; Kincaid 1983; Leary et al. 1985; Allendorf and Ryman 1987).

A growing body of empirical evidence suggests that heterozygotes at protein coding loci have greater values for components of fitness such as growth rate, developmental stability, fertility, and viability (Mitton and Grant 1984; Allendorf and Leary 1986; Zouros and Foltz 1987; Quattro and Vrijenhoek 1989) than homozygotes at these loci. Positive associations between heterozygosity and size, developmental rate, and developmental stability have been found in several hatchery strains of salmonids (Danzmann et al. 1986, 1987, 1988; Ferguson et al. 1985; Leary et al. 1983, 1984, 1985). More heterozygous females also have greater values for components of fecundity (i.e. egg size, egg number) in a hatchery strain of rainbow trout (*Oncorhynchus mykiss*) (Danzmann et al. 1989) and a naturalized population of brook trout (*Salvelinus fontinalis*) (Liskauskas and Ferguson 1990) than less heterozygous females.

The current study examines the relationship between heterozygosity and several components of fitness, namely size and survival, in a naturalized population of brook trout from Mykiss Lake, Algonquin Provincial Park, Ontario. Individuals were sampled during several life history stages, including emergence from redd sites and at the onset of sexual maturation, periods in which survival and growth differences between heterozygotes and homozygotes may be most pronounced due to high mortality or putative stress (Koehn and Shumway 1982; Gaffney and Diehl 1986; Allendorf and Leary 1986). The popula-

tion studied was established from the planting of several brook trout strains (Fraser 1989). This diverse source of potential genetic variation provided an opportunity to examine the relationship between heterozygosity and several components of fitness in individuals that have been exposed to the selective pressures of the natural environment.

Materials and Methods

Origin of Fish

The experimental fish were collected from Mykiss Lake (45°41′N, 78°14′W), Algonquin Park, Ontario, during the spring, summer, and fall of 1988 and in June 1990. Mykiss Lake (Fig. 1) is a small (23.5 ha) mesotrophic, softwater lake. The lake was designated as a fish sanctuary for research purposes by the Ontario Ministry of Natural Resources in 1962 and stocked with domestic Hill's Lake Hatchery strain, wild Dickson Lake (endemic to Algonquin Park), and Lake Nipigon strains. A chronological history of these introductions is outlined in Fraser (1989). The lake was treated with a fish toxicant in 1965 to remove all fish (primarily white suckers, *Catostomus commersoni*) (Jim Fraser, Ontario Ministry of Natural Resources, Maple, Ont., pers. comm.). Since 1975, naturally reproduced brook trout began appearing in gillnet surveys (Fraser 1989). The present population consists of naturally reproduced individuals, and approximately two generations (6 yr) have passed since the last introductions.

From May 4 to 6, 1988, 69 brook trout young of the year (YOY May) were collected with hand-held dip nets from depths ranging from 0.2 to 1 m from nearshore areas located in close proximity to the only known brook trout spawning shoal (Fig. 1). Once captured, individuals were killed with a fish anaesthetic (tricaine methanesulfonate), measured (nearest millimetre), and stored in liquid nitrogen.

From June 8 to 13, 1988, brook trout young of the year (YOY June), yearlings (1 yr), and 2-yr-old individuals were collected utilizing a variety of fish capture gear. A survey of nearshore areas revealed that YOY were dispersed throughout most of the perimeter of the lake. Plexiglas trap nets and nylon trap nets were randomly set in nearshore areas around the perimeter of the lake (Fig. 1) and a sample of 89 YOY was collected, killed, measured (nearest millimetre), and stored in liquid nitrogen. A sample of forty 1-yr-old and thirty-three 2-yr-old fish was collected from the previously mentioned trap net sets as well as by angling with rod and reel. These individuals were killed, sexed, measured (nearest millimetre), and weighed (nearest gram) before tissue samples (eye, liver, and muscle) were removed and stored in liquid nitrogen. YOY from both the May and June collections were subsequently weighed (nearest 0.1 mg) prior to further analysis.

On October 31, 1988, a sample of fifty-two 3-yr-old and ten 4-yr-old brook trout were collected from trap nets set over the spawning shoal (Fig. 1). Individuals were killed, sexed, measured (nearest millimetre), and weighed (nearest gram). Tissue samples (eye, liver, and muscle) were removed and stored in liquid nitrogen.

To trace the progress of the YOY cohort sampled in May and June of 1988, a sample of thirty-five 2-yr-olds was collected in June of 1990 using procedures identical to those in 1988. Ages of all fish except YOY were determined from scales and otoliths as described by Liskauskas and Ferguson (1990).

To determine recent growth rates of brook trout sampled at various times of the year, a technique which is based on the amount of RNA relative to DNA in white muscle was used (Bulow 1987). Fish experiencing high growth rates are characterized by having higher RNA concentrations and RNA:DNA ratios than fish growing at slower rates. The concentration of RNA relative to DNA in white muscle was used to determine recent growth according to methods of Karsten and Wollenberger (1972, 1977) as modified by Ferguson and Drahushchak (1989).

Genetic Analysis

Samples of eye, liver, and white muscle tissue collected in the field were transported in liquid nitrogen to laboratory facilities at the University of Guelph for electrophoretic analysis. Tissue samples from YOY were sampled in the laboratory. Procedures for tissue extraction, horizontal starch gel electrophoresis, and staining techniques followed those described previously (Utter et al. 1974; Allendorf et al. 1977; May et al. 1979). The enzymes examined in this study, their abbreviations, International Union of Biochemistry enzyme classification numbers (EC), the loci that code for them, and the tissues used are listed in Table 1. Allelic nomenclature followed that of Allendorf and Utter (1979) with modifications by May et al. (1979).

Statistical Analysis

The phenotypic distribution observed at each polymorphic locus was tested for departure from Hardy–Weinberg proportions using a goodness of fit chi square (χ^2) test with Yates correction for small sample size. A contingency χ^2 analysis was used to test for homogeneity of allelic counts among age classes and between sexes within each age class (individuals aged 1–4 yr). The treatment of duplicated loci (*Aat-1,2* and *Mdh-3,4*) followed that of Liskauskas and Ferguson (1990).

Several methods were used to test for statistically significant genetic differences among age classes. Since the samples of YOY were based on the same cohort, they were analyzed separately from the other age classes. A Mann–Whitney U test (Sokal and Rohlf 1981) was used to determine if the observed number of heterozygous loci per individual differed between the May and June samples as well as the June 1990 sample. The May sample of YOY was not scored for *G3p-1* because of poor resolution of enzyme activity and this locus was not included in the latter analysis. Eleven polymorphic loci were common to the June sample of YOY as well as the remaining age classes. Therefore, these samples were compared for differences in the number of heterozygous loci per individual using a Tukey HSD multiple mean comparison test.

The associations between multilocus heterozygosity (number of heterozygous loci per fish) or single locus heterozygosity and fish length, fish weight, and RNA:DNA ratios were determined using linear regression analysis as described by Liskauskas and Ferguson (1990). The regression analysis was conducted separately for each sex for age classes where individuals were sexed (age classes 1–4 inclusive).

Results

Genetic Variation

Allozyme variation

Polymorphisms were detected at 12 out of 44 loci analyzed in individuals from most age classes (Table 2). No significant

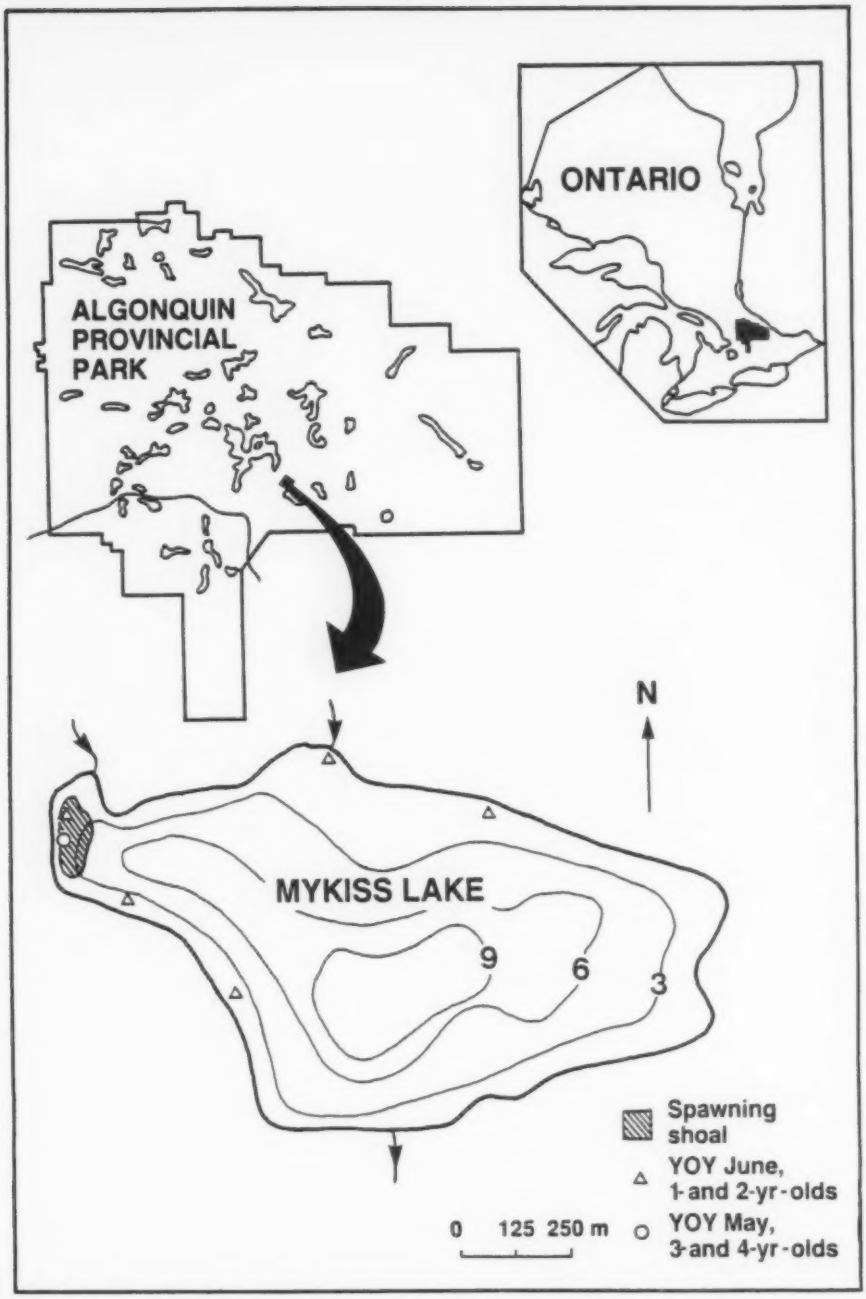

FIG. 1. Locations of brook trout sampling areas and the spawning shoal in Mykiss Lake, Algonquin Park, Ont. YOY refers to young of the year collected in May or June. Depth contours are in metres.

deviations for Hardy–Weinberg proportions were found within each age class, nor in all age classes when pooled (χ^2 goodness of fit).

Allele frequency among age classes

Of the 15 comparisons of allele counts between sexes within an age class, only one significant difference ($\chi^2 = 4.82$; $P < 0.05$) between 3-yr-old males and females at *G3p-1* was found. This is less than that expected by chance alone. Subsequent comparisons of allele counts were therefore based on data summed across sex. Age classes differed in allele counts at only one locus (*Aco-1*, $P < 0.001$; Table 1) out of six for which the most common allele occurred at a frequency of less than 95%. In addition, the two YOY samples differed significantly for allele counts at two loci (*Aat-1,2*, $\chi^2 = 4.086$, $P = 0.043$; *Aco-1*, $\chi^2 = 4.547$, $P = 0.033$). No significant differences in allele counts were found between the YOY sampled in June of

TABLE 1. Enzymes, International Union of Biochemistry enzyme classification numbers (EC), loci, and tissues of expression for brook trout from Mykiss Lake, Algonquin Park, Ont.

Name	EC No.	Loci	Tissue
Acid phosphatase	3.1.3.2	*Acp-1* *Acp-2*	Liver
Aconitase	4.2.1.3	*Aco-1**	Liver
Adenylate kinase	2.7.4.3	*Ak-1* *Ak-2*	Muscle
Alcohol dehydrogenase	1.1.1.1	*Adh*	Liver
Aspartate aminotransferase	2.6.1.1	*Aat-1,2** *Aat-3,4*	Muscle Liver
Creatine kinase	2.7.3.2	*Ck-1* *Ck-2** *Ck-3*	Muscle
Fumerase	4.2.1.2	*Fh-1,2*	Liver
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	*Gapdh*	Muscle
Glycerol-3-phosphate dehydrogenase	1.1.1.8	*G3p-1**	Muscle
Glucosephosphate isomerase	5.3.1.9	*Gpi-1** *Gpi-2* *Gpi-3*	Muscle
Glutamic pyruvic transaminase	2.6.1.2	*Gpt*	Muscle
β-Glucoronidase	3.2.1.31	*Gus*	Liver
Hexosaminidase	3.2.1.30	*Hex*	Liver
Isocitrate dehydrogenase	1.1.1.42	*Idh-3** *Idh-4*	Liver
Lactate dehydrogenase	1.1.1.27	*Ldh-1,2* *Ldh-3** *Ldh-4**	Muscle Eye Liver
Malate dehydrogenase	1.1.1.37	*Mdh-1,2* *Mdh-3,4**	Eye Muscle
Malic enzyme	1.1.1.40	*Me-1** *Me-2*	Muscle
Mannosephosphate isomerase	5.3.1.8	*Mpi*	Eye
Phosphoglucomutase	5.4.2.2	*Pgm-1**	Muscle
Phosphogluconate dehydrogenase	1.1.1.44	*Pgd**	Muscle
Phosphoglycerate kinase	2.7.2.3	*Pgk-1* *Pgk-2*	Liver
Pyruvate kinase	2.7.1.40	*Pk-1*	Muscle
Sorbitol dehydrogenase	1.1.1.14	*Sdh-1* *Sdh-2*	Liver
Superoxide dismutase	1.15.1.1	*Sod-1*	Muscle
Xanthine dehydrogenase	1.1.1.20	*Xdh*	Liver

NOTE: Loci separated by commas are isoloci whose protein products are electrophoretically indistinguishable. These pairs are therefore considered as single tetrasomic loci in all analysis. * Loci which were polymorphic in this study.

1988 and 2-yr-olds sampled in June of 1990. Changes in allele counts between different age classes were further indicated by significant regressions between the frequency of the most common allele (arcsine transformed) and fish age for two loci (*Aco-1*, $R^2 = 0.769$, $F_{(1,4)} = 13.388$, $P < 0.05$; *G3p-1*, $R^2 = 0.89$, $F_{(1,3)} = 24.337$, $P < 0.05$). The frequency of the most common allele for both these loci decreased significantly as the age of the fish increased.

Multilocus Heterozygosity and Age

Individuals sampled at different dates as well as from different age classes differed in the number of heterozygous loci per fish. YOY sampled in May had significantly fewer heterozygous loci per individual ($P < 0.05$, Mann–Whitney U test) than YOY sampled in June (Fig. 2). However, the number of heterozygous loci per individual for the 2-yr-olds sampled in

TABLE 2. Allele frequencies in five age classes of brook trout from Mykiss Lake, Algonquin Park, Ont.

Locus	Allele	Age class: YOY May (69)	YOY June (89)	1 (40)	2 (33)	3 (52)	4 (10)	df	X^2
Aat-1,2	100	0.924	0.888	0.888	0.909	0.913	0.875	5	6.025
	118	0.076	0.112	0.112	0.091	0.087	0.125		
Aco-1	100	0.964	0.893	0.888	0.924	0.779	0.700	5	27.914***
	67	0.036	0.107	0.112	0.076	0.221	0.300		
Ck-2	100	1.000	1.000	1.000	0.970	0.962	1.000		
	122				0.030	0.038			
G3p-1	100	—	0.796	0.775	0.758	0.692	0.650	4	3.192
	167	—	0.204	0.225	0.242	0.308	0.350		
Gpi-1	100	0.993	1.000	1.000	0.985	0.971	1.000		
	125	0.007			0.015	0.029			
Idh-3	100	0.848	0.848	0.863	0.833	0.942	0.800	10	7.309
	128	0.051	0.078	0.037	0.046		0.150		
	150	0.101	0.074	0.100	0.121	0.058	0.050		
Ldh-3	100	0.971	0.938	0.963	0.985	1.000	1.000		
	72	0.029	0.062	0.037	0.015				
Ldh-4	100	0.971	1.000	0.950	0.985	1.000	1.000		
	200	0.029		0.050	0.015				
Me-1	100	0.949	0.916	0.888	0.864	0.875	0.900	5	6.135
	0	0.051	0.084	0.112	0.136	0.125	0.100		
Mdh-3,4	100	0.982	0.978	0.981	0.977	0.976	0.950		
	120	0.011	0.017	0.019	0.015	0.024	0.050		
	60	0.007	0.006		0.008				
Pgd	100	0.993	0.989	0.950	0.985	0.981	1.000		
	86	0.007	0.011	0.050	0.015	0.019			

NOTE: Sample size for each age class in parentheses. Contingency X^2 are for the comparison of allele counts among age classes. ***$P < 0.001$.

June of 1990 (representing the YOY cohort after 2 yr in the wild) did not differ significantly ($P > 0.10$, Mann–Whitney U test) from the YOY collected in May or June of 1988 (Fig. 3). For the remaining age classes sampled in 1988, individuals from age classes 1 and 2 had significantly fewer heterozygous loci per individual ($P < 0.01$, Tukey HSD test) than individuals in age class 4 (Fig. 2). No significant differences in the number of heterozygous loci per individual ($P > 0.10$ in all cases) were found among the June sample of YOY, 3-yr-olds, and 4-yr-olds. Although there seemed to be a general increase in the mean number of heterozygous loci per fish for individuals aged 1 or above, this trend was not statistically significant based on regression analysis.

Heterozygosity, Size, and Growth

Fish weight

All loci summed — Significant linear relationships were found between multilocus heterozygosity and fish weight for 3- and 4-yr-olds only. In the 3-yr-old age class, females which were more heterozygous were heavier than those which were less heterozygous ($F_{(1,25)} = 3.836$, $P_{1\ tail} < 0.05$, $R^2 = 0.13$; Fig. 4). Although sample sizes were small for the 4-yr-old age class, a similar trend was also noted; 4-yr-old females that were more heterozygous were heavier than less heterozygous females ($F_{(1,2)} = 15.474$, $P_{1\ tail} < 0.05$, $R^2 = 0.89$). Four-year-old males, however, showed the opposite pattern in that males which were more heterozygous were lighter than those which were less heterozygous ($F_{(1,4)} = 10.949$, $P_{1\ tail} < 0.05$, $R^2 = 0.73$). No significant linear relationships between multilocus heterozygosity and fish weight were found within the remaining age classes.

Single locus effects — Several loci accounted for a significant amount of variation in fish weight for 3-yr-old females and males (Table 3). The mean weights of females heterozygous at *G3p-1* or *Idh-3* were greater than that for homozygotes whereas females heterozygous at *Ldh-3* were lighter on average than homozygotes (Table 4). For the 3-yr-old males, individuals heterozygous at *Aat-1,2* were on average heavier than homozygotes at the respective loci (Table 4). Only a single locus (*Aat-1,2*) accounted for a significant amount of variation in fish weight for 4-yr-old males. The mean weight of males heterozygous at *Aat-1,2* was less than that of homozygotes. No single locus accounted for a significant amount of variation in weight for 4-yr-old females as well as in the remaining age classes.

Fish length

All loci summed — Three-year-old males displayed a significant negative linear relationship between multilocus heterozygosity and fish length ($F_{(1,23)} = 4.341$, $P_{1\ tail} < 0.05$, $R^2 = 0.16$; Fig. 4). In the 4-yr-old age class, females which were more heterozygous were longer than less heterozygous females ($F_{(1,2)} = 24.747$, $P_{1\ tail} < 0.05$, $R^2 = 0.93$) whereas males which were more heterozygous were shorter than less hetero-

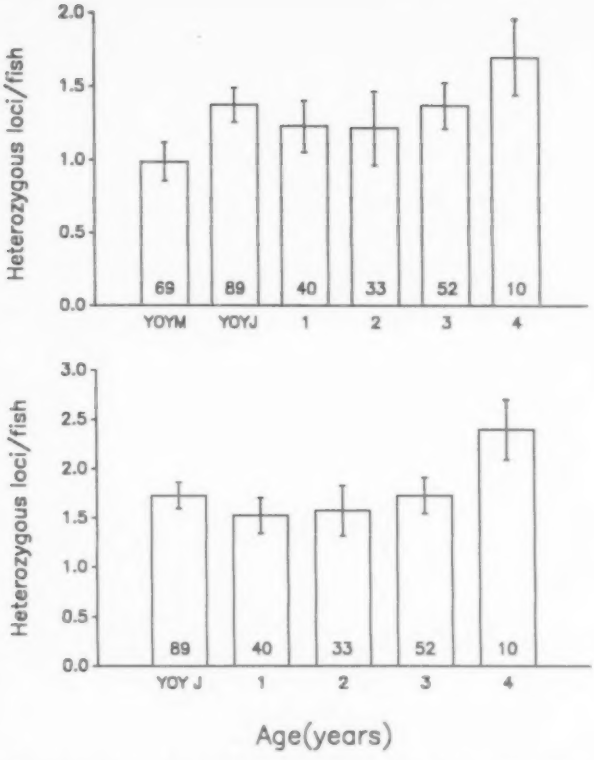

FIG. 2. Mean number of heterozygous loci per fish for five age classes of brook trout from Mykiss Lake, Algonquin Park, Ont. Means for the upper panel were based on 10 polymorphic loci common to all age groups. Means for the lower panel were based on 11 polymorphic loci common to all age classes except for the YOY May sample. Sample sizes are given for each age class. Vertical lines represent twice the standard error of the means. YOY refers to young of the year sampled in May and June of 1988.

zygous males ($F_{(1,4)} = 7.228$, $P_{1\text{ tail}} < 0.05$, $R^2 = 0.64$). For the YOY sampled in June, more heterozygous individuals were marginally longer ($F_{(1,87)} = 2.651$, $P_{1\text{ tail}} = 0.054$, $R^2 = 0.03$) than less heterozygous individuals.

Single locus effects — The significant single locus effects for fish length in the 3- and 4-yr-olds were identical to those described previously for fish weight (Table 3). Three-year-old females heterozygous at *G3p-1* or *Idh-3* were on average longer than homozygotes at these loci whereas females heterozygous at *Ldh-3* were on average shorter than homozygotes (Table 4). As with male weight, the mean length of 3-yr-old males heterozygous at *Aat-1,2* was greater than the mean length of homozygotes, and males heterozygous at *G3p-1*, *Idh-3*, or *Aco-1* were on average shorter than homozygotes at these respective loci (Table 4). The mean length of 4-yr-old males heterozygous at *Aat-1,2* was less than that for homozygous males at this locus (Table 4). For 2-yr-old males, heterozygotes at *Me-1* were on average longer than homozygotes (Table 4). Two significant single locus effects were found for the YOY sampled in June. Individuals heterozygous for *Me-1* or *Ldh-3* were on average longer than homozygotes at these respective loci (Table 4).

Recent growth

The two age classes (3- and 4-yr-olds) collected in October 1988 did not differ significantly from each other for mean

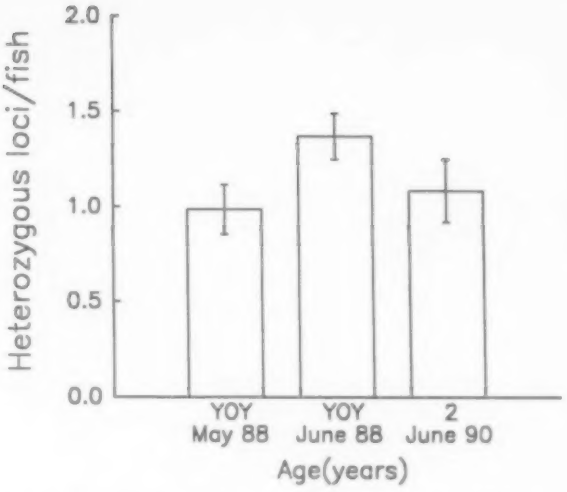

FIG. 3. Mean number of heterozygous loci per fish for the YOY cohort sampled in 1988 and in 1990 from Mykiss Lake, Algonquin Park, Ont. Vertical lines represent twice the standard error of the means.

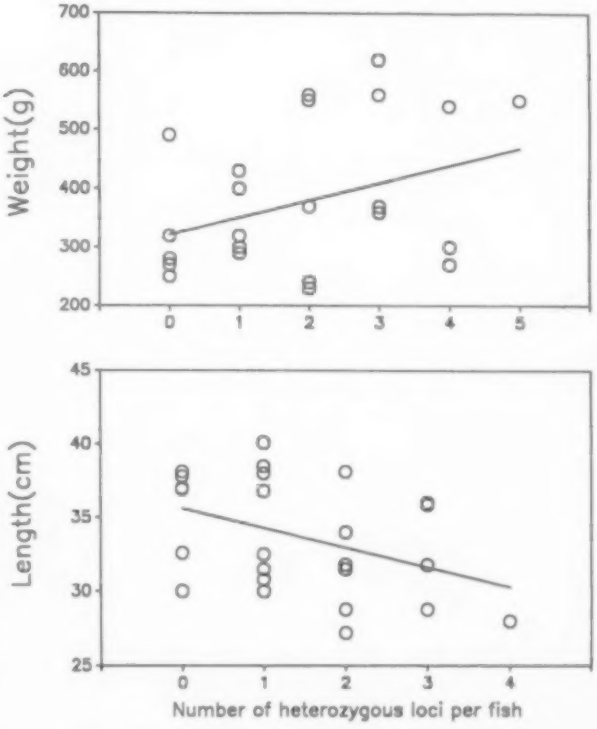

FIG. 4. Regression of fish weight on the number of heterozygous loci per 3-yr-old female brook trout ($N = 27$) and of fish length on the number of heterozygous loci per 3-yr-old male brook trout ($N = 25$) from Mykiss Lake, Algonquin Park, Ont.

TABLE 3. Single loci contributing to the relationship between fish weight, fish length, and heterozygosity for brook trout from Mykiss Lake, Algonquin Park, Ont.

Dependent variable	Age class	Sex	Locus	F (df)	P
Fish weight	4	Male	*Aat-1,2(−)*	17.333 (1,4)	0.015
	3	Male	*Aat-1,2(+)*	5.842 (1,20)	0.025
			G3p-1(−)	7.092 (1,20)	0.015
			Aco-1(−)	6.870 (1,20)	0.016
			Idh-3(−)	5.072 (1,20)	0.036
	3	Female	*Idh-3(+)*	10.407 (1,23)	0.004
			G3p-1(+)	8.088 (1,23)	0.009
			Ldh-3(−)	8.994 (1,23)	0.006
Fish length	4	Male	*Aat-1,2(−)*	10.258 (1,4)	0.033
	3	Male	*Aat-1,2(+)*	8.644 (1,20)	0.008
			G3p-1(−)	16.209 (1,20)	0.001
			Idh-3(−)	9.054 (1,20)	0.007
			Aco-1(−)	8.738 (1,20)	0.008
	3	Female	*Idh-3(+)*	6.687 (1,23)	0.017
			G3p-1(+)	6.012 (1,23)	0.022
			Ldh-3(−)	6.938 (1,23)	0.015
	2	Male	*Me-1(+)*	4.978 (1,18)	0.039
	0 June	—	*Me-1(+)*	6.729 (1,87)	0.011
			Ldh-3(+)	4.056 (1,87)	0.047

NOTE: Locus partial regression coefficients in parentheses. A positive partial regression coefficient indicates that the heterozygote is associated with greater weight or length whereas a negative partial regression coefficient indicates that the homozygote is associated with the greater weight or length. 0 June referes to the YOY sampled in June. Information on sex was not available for the YOY.

RNA:DNA ratio (t-test (2 tail), $P > 0.10$). Three-year-old males had marginally lower RNA:DNA ratios (t-test (2 tail), $P = 0.063$) than 3-yr-old females.

There was insufficient evidence from the regression analysis that multilocus heterozygosity was associated with recent growth rate as estimated by RNA:DNA ratios. However, more heterozygous 3-yr-old males had marginally lower RNA:DNA ratios ($F_{(1,19)} = 2.906$, $R^2 = 0.133$, P (1 tail) $= 0.053$) than less heterozygous males. Several individual loci accounted for a significant amount of variation in RNA:DNA ratios in these males. Males heterozygous at *Aco-1* and *Ck-2* had lower RNA:DNA ratios than homozygotes (partial $F_{(1,17)} = 19.927$, $P < 0.001$; partial $F_{(1,17)} = 7.667$, $P < 0.05$, respectively) whereas males heterozygous at *Idh-3* had significantly higher RNA:DNA ratios than homozygotes (partial $F_{(1,17)} = 5.842$, $P < 0.05$).

Discussion

Temporal Changes in Genetic Variation

The amount of genetic variation differed among age classes spanning most of the life-history stages of brook trout in this population. Although there was no evidence of departures from Hardy–Weinberg proportions to suggest population subdivision or differential strain contributions, significant variation in allele frequencies and number of heterozygous loci per individual were found. Differences in allele frequencies between age classes may have been the result of changes caused by natural selection as fish grew older. Significant regressions between allele frequencies and age suggest that some of the loci examined, or loci in linkage disequilibrium with the enzyme loci, affect survival. Many studies on invertebrates and vertebrates have noted an increase in heterozygosity at enzyme loci with age (reviewed in Allendorf and Leary 1986; Zouros and Foltz 1987). These increases in heterozygosity with age have been attributed to possible viability gains among older heterozygotes, not seen in younger ages because selection has yet to act upon them. Although individuals from the oldest age class examined in this study were the most heterozygous, a statistically significant trend for increased heterozygosity over time was not evident. Making inferences on the selective advantage of heterozygosity over time is complicated because environmental conditions and the genetic background between year classes may differ from year to year.

Tracing the progress of a cohort through time avoids most of the complications just mentioned. Significant differences in both allele frequencies and the number of heterozygous loci per individual were found in the YOY cohort. YOY sampled in June had significantly more heterozygous loci per individual than YOY sampled in May. These findings provide some evidence of the selective advantage of heterozygosity.

Many studies of brook trout population dynamics point to high mortalities during the first summer of life (Smith 1947; Cooper 1953; McFadden 1961; Latta 1962). Latta (1968) provided evidence that starvation was a probable cause of mortality, with decreased resistance to starvation as temperatures increased. The mortality factor is important to consider when comparing the two samples of YOY. The initial sample collected in May consisted of individuals that were still relying on yolk reserves for nutrition. However, the second sample of YOY was collected at a time when exogenous feeding was necessary as a result of yolk supply depletion. Since YOY brook trout are highly territorial in rivers (Grant and Noakes 1988; McNicol and Noakes 1984), and most likely in lakes as well, any advantages conferred by increased heterozygosity

TABLE 4. Mean weights (g) and lengths (cm) with standard errors (in parentheses) of homozygotes and heterozygotes at enzyme loci accounting for a significant amount of variation in brook trout weight or length within each of the brook trout age classes.

Dependent variable	Locus and genotypes	Sex	Age class			
			4	3	2	YOY June
Weight	*Aat-1,2*					
	Homozygotes	Males	430 (20.0) (2)	411.1 (28.7) (19)		
	Heterozygotes		495 (6.5) (4)	471.7 (48.7) (6)		
	Aco-1					
	Homozygotes			453.9 (31.2) (18)		
	Heterozygotes			352.9 (21.9) (7)		
	G3p-1					
	Homozygotes			433.7 (28.8) (19)		
	Heterozygotes			400.0 (51.5) (6)		
	Homozygotes	Females		355.7 (25.6) (14)		
	Heterozygotes			428.5 (33.0) (13)		
	Idh-3					
	Homozygotes	Males		433.2 (25.8) (22)		
	Heterozygotes			370.0 (92.9) (3)		
	Homozygotes	Females		366.7 (21.3) (24)		
	Heterozygotes			490.0 (97.1) (3)		
	Ldh-3					
	Homozygotes			383.5 (22.9) (26)		
	Heterozygotes			300.0 (−) (1)		
Length	*Aat-1,2*					
	Homozygotes	Males	36.3 (0.4) (4)	33.4 (0.9) (19)		
	Heterozygotes		33.7 (0.9) (2)	34.8 (1.5) (6)		
	Aco-1					
	Homozygotes			34.5 (1.5) (18)		
	Heterozygotes			31.8 (0.5) (7)		
	G3p-1					
	Homozygotes	Males		34.3 (0.8) (19)		
	Heterozygotes			31.9 (1.7) (6)		
	Homozygotes	Females		31.4 (0.7) (14)		
	Heterozygotes			33.8 (1.0) (13)		
	Ldh-3					
	Homozygotes	Males		34.0 (0.8) (22)		
	Heterozygotes			31.7 (2.5) (3)		
	Homozygotes	Females		32.2 (0.6) (24)		
	Heterozygotes			35.0 (2.6) (3)		
	Ldh-3					
	Homozygotes			32.6 (0.7) (26)		5.2 (0.1) (78)
	Heterozygotes			29.9 (−) (1)		5.8 (0.3) (11)
	Me-1					
	Homozygotes	Males			25.3 (0.4) (4)	5.2 (0.1) (74)
	Heterozygotes				27.4 (0.6) (16)	5.9 (0.2) (15)

NOTE: Numbers in parentheses below means indicate the sample sizes. YOY sampled in June were not sexed.

would be most beneficial at this time. A continued increase in the mean number of heterozygous loci per individual over time might be expected if a selective advantage exists for increased heterozygosity. The sample of 2-yr-olds collected in June of 1990 was expected to be more heterozygous than the previous samples. The fact that this was not the case may be a result of the small sample size precluding the detection of variant alleles present in low frequencies.

Heterozygosity and Size (YOY)

A weak but positive association between heterozygosity and size was found in YOY at a critical time when individuals had made the transition from endogenous to exogenous modes of feeding. More heterozygous YOY sampled in June were longer than less heterozygous YOY. Since this association was only found in the June sample, it provides some support to the hypothesis that associations between heterozygosity and size are most likely to be detected during periods of low energy balance and putative stress (Koehn and Shumway 1982; Gaffney and Diehl 1986). During early development, production is dominated by growth and one would expect that associations between heterozygosity and growth would be most easily detected because individual differences in energy balance are predominantly manifested as differences in growth rate (Koehn 1990). It appears that the onset of exogenous feeding and the highly territorial nature of young brook trout provide such conditions. The benefits incurred from being larger at this point in the life cycle are many. Several studies on a variety of salmonid species have found that larger individuals are less susceptible to starvation (Adelman et al. 1955; Bagenal 1969) and show higher levels of agonistic behaviour (Wankowski and Thorpe 1979; Ferguson and Danzmann 1985; Ferguson et al. 1988). Therefore, more heterozygous individuals may have an advantage in establishing and defending more optimal territories because they are larger.

Heterozygosity and Size at Sexual Maturity

The association between heterozygosity and size at the time of sexual maturation differed according to sex in brook trout from this population. Positive associations were detected for mature females whereas the opposite was true for mature males. Differences in the direction of associations between heterozygosity and growth with age have been previously found in other salmonid populations (Danzmann et al. 1987, 1988; Ferguson 1990). Danzmann et al. (1987, 1988) observed positive associations in rainbow trout before 6 mo of age whereas Ferguson (1990) observed negative associations when sexual maturity was likely to be a factor. Ferguson (1990) suggested that males which were more heterozygous were growing faster prior to sexual maturity and thus maturing at an earlier time than males which were less heterozygous. Early sexual maturation of 1-yr-old rainbow trout is associated with rapid growth prior to this maturation (Gall 1985). If one assumes that energy reserves in individuals are finite, males that are maturing earlier are investing more energy toward gamete production and therefore less energy would be available for growth. Further support of this argument is offered by RNA:DNA ratios. Three-year-old male brook trout had lower RNA:DNA ratios than 3-yr-old females. As well, more heterozygous 3-yr-old males had marginally lower RNA:DNA ratios than less heterozygous males. Thus, it appears that more heterozygous males were growing more slowly at the time of collection.

The previous explanation does not fully explain the difference in the direction of the regressions for heterozygosity and size in male and female brook trout. It may be that the allocation to somatic tissue elaboration is different between the sexes at maturation. The higher RNA:DNA ratios for females over males support this hypothesis. Previous studies have found that energetic investments into ovaries are greater than for testes (Wootton and Evans 1976; Beamish 1979) and that the allocation of energy toward testes production is usually small and cannot explain the decrease in growth of male fish with the onset of maturity (Roff 1983). The results from this study seem to indicate that males are more taxed at the onset of maturity than females. Several arguments could be put forward to explain these differences. The age composition of males collected from the spawning beds in October indicated that most of the males were 3 yr of age, but there were a few mature 2-yr-old males. Mature 2-yr-old males that were more heterozygous were not lighter ($F_{(1,5)} = 0.045$, $P > 0.10$), not shorter ($F_{(1,5)} = 0.860$, $P > 0.10$), and did not have lower RNA:DNA ratios ($F_{(1,5)} = 1.889$, $P > 0.10$) than less heterozygous mature males; however, in all cases the regression coefficients were negative. There were no mature 2-yr-old females collected from the spawning beds. Since males mature earlier than females, the 3- and 4-yr-old males may have had the potential to have spawned in more than one year. This repeated investment toward reproduction may have a cumulative effect in decreasing a male's ability to maintain energy reserves for growth at the time of reproduction. An increased investment in reproductive relative to somatic growth with age seems to be a general trend among adult iteroparous fish species until they reach senility (Nikolsky 1963). In addition to these repeated demands on energy reserves, males also become involved in intense competition for mates, a trait commonly displayed in salmonids (Hanson and Smith 1967).

The positive association between heterozygosity and size in females may indicate that females are more efficient or utilize different strategies for diverting energy toward gamete production than males. Females appear to mature at a later age than males in this population of brook trout. Since fecundity is generally an increasing function of body size (Bagenal 1966; Wootton 1979), females may be showing restraint in gonadal production, thus reducing postspawning mortality and increasing future growth and fecundity (Jonsson et al. 1988). In fish, fecundity generally increases with size and hence, all other things being equal, there are selective pressures for continued growth of females (Roff 1983). Another complicating factor is that female size includes both the somatic and gametic tissue components. The heterozygosity/growth relationship may be complicated by the fact that more heterozygous females may be more efficient in directing energy reserves towards gametic tissue elaboration. This possibility becomes even more likely due to the fact that heterozygosity was positively associated with female weight (which includes somatic and gametic tissue) and not female length which is a better indicator of cumulative growth effects. A previous detailed study of the gametic products from 3-yr-old females used in this study revealed that more heterozygous females have heavier ovulated egg compliments as well as having a greater number of ovulated eggs than less heterozygous females (Liskauskas and Ferguson 1990).

Conserving Genetic Variation

Biochemical genetic variation is associated with traits likely to be important in the survival of stocked fish. It is also well

recognized that the loss of genetic variation from a brood stock can adversely affect important characteristics such as survival, growth, feed conversion, body shape, and normal development (reviewed by Kirpichnikov 1981; Kincaid 1983; Leary et al. 1985). Although this study was not designed to distinguish between the genetic mechanisms responsible for the association between heterozygosity and components of fitness, it does provide support for the differential effects that time and age of sampling can have towards this relationship. Heterozygosity was positively associated with several phenotypic effects that are likely to affect an individual's overall fitness. The potential sources of genetic variation available towards the establishment of this naturalized population may be one of the contributing factors to its success. The stocking of both wild and hatchery strains into Mykiss Lake may have provided the genetic resources necessary to establish a viable and naturally sustained population.

Acknowledgements

We especially thank Dr. Leon Carl, Jim Fraser, and Greg Betteridge of the Harkness Laboratory for Fisheries Research, Algonquin Park, Ont., for their logistical and technical support of our field work. Collection of field data was accomplished with the able assistance of Lenore Drahushchak, Stephen Gile, and Wendylee Stott. We also thank Dr. Peter Ihssen and Bill Martin for their advice on electrophoretic procedures and Dan Ryan for his helpful advice on statistical analysis. This research was supported by funds from NSERC and a Renewable Resources Research Grant from the Ontario Ministry of Natural Resources.

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Importance of Threshold Length and Photoperiod for the Development of Bimodal Length–Frequency Distribution in Atlantic Salmon (*Salmo salar*)

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Juveniles of 0+ Atlantic salmon (*Salmo salar*) were graded in narrow length intervals to test the hypothesis that a threshold length determines the segregation into bimodal length–frequency distributions. In the experiment, 1350 juveniles of an initial length of 70–80 mm were held under different light and temperature conditions. Distinct decreases in temperature for short or long periods did not influence the degree of bimodal segregation and temperature did not, thus, seem to be a winter stimulus for segregation in fish of this size. Transfer of fish from continuous light to natural photoperiod was followed by a segregation in growth rates into lower and upper mode fish. Upper mode fish developed smolt appearance the following spring. Under continuous light the bimodality was weak or absent and these fish did not develop morphological characteristics typical of smolts. It is concluded that the individual decision to enter the upper mode and undergo silvering the following spring is strongly dependent on the length of the fish at the time of the winter light stimulus. Fish smaller than a threshold length of about 75 mm reduce their growth rate in response to the natural photoperiod, while fish above this size enter the upper length–frequency mode.

On a réparti des juvéniles de saumon de l'Atlantique (*Salmo salar*) de 0+ an selon de petits intervalles de longueur afin de vérifier l'hypothèse selon laquelle un seuil de longueur détermine la ségrégation en distributions bimodales de fréquence des longueurs. L'expérience était basée sur l'élevage dans diverses conditions d'éclairage et de température de 1 350 juvéniles mesurant de 70 à 80 mm de longueur. Des baisses marquées de la température pendant de courtes ou de longues périodes n'ont pas influé sur le niveau de ségrégation bimodale; la température ne semble donc pas être un stimulus de la ségrégation du poisson de cette taille en hiver. Le transfert des saumons exposés à un éclairage continu à un milieu à photopériode naturelle a été suivi d'une ségrégation des taux de croissance en modes inférieur et supérieur. Les juvéniles de cette deuxième catégorie se sont transformés en saumoneaux le printemps suivant. Dans des conditions d'éclairage continu, le caractère bimodal était faible ou absent; ces poissons n'ont pas présenté les caractéristiques morphologiques typiques des saumoneaux. On formule la conclusion que l'atteinte individuelle du mode supérieur et donc le développement d'une robe argentée le printemps suivant dépendent fortement de la longueur du poisson au moment où se présente le stimulus de l'éclairage hivernal. Les poissons de longueur inférieure au seuil de longueur d'environ 75 mm présentaient une diminution de leur taux de croissance en réaction à la photopériode naturelle, tandis que celui des poissons de plus de 75 mm de longueur correspondait au mode supérieur de fréquence des longueurs.

Received July 13, 1989
Accepted May 1, 1991
(JA224)

Reçu le 13 juillet 1989
Accepté le 1 mai 1991

When reared in hatcheries, juveniles of Atlantic salmon (*Salmo salar*) may develop a bimodal length–frequency distribution during their first year of life. The upper mode contains those fish which will complete smoltification the following spring (Thorpe 1977; Thorpe et al. 1980; Bailey et al. 1980), a development pattern which is probably common for rapidly growing river populations (Bagliniére and Maisse 1985; Bagliniére and Champigneulle 1986).

Several attempts have been made to explain how and when this length segregation occurs. Simpson and Thorpe (1976) proposed a relationship between falling temperature in autumn, increase in thyroxine because of decreasing daylength, and differences in temperature-dependent thyroxin-response levels between potential lower and upper mode fish. They suggested that potential upper mode fish with a low thyroxine response level may continue growth under low temperatures, while the growth of fish with a high thyroxine response level ceases. Metabolic rates have also been found to differ between the two modal groups (Thorpe et al. 1982). Recently, the growth patterns of the two groups have been more thoroughly studied. The lower mode fish lose both appetite and growth rate (Metcalfe et al. 1986, 1988), while the upper mode fish enter an interval of faster growth (Kristinsson et al. 1985; Skilbrei 1988). Metcalfe et al. (1988) proposed that fish that have an increased appetite in autumn adapt a fast development strategy whereas those fish that show a reduction in appetite become slow developers. Villarreal et al. (1988) found support for a model in which growth is maintained if feeding is above a threshold level and in which this critical timing is influenced by photoperiod.

Elson (1957) suggested that parr that reach a length of about 10 cm at the end of the growing season would probably be able

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to smoltify the following spring. It has been suggested, although no proof was given, that the threshold length may be a regulating factor in the development of bimodality (Skilbrei 1988). Threshold length may mark the entrance to the period of faster growth for fish reaching this length at an appropriate time of the year, or the continuation of low growth for fish reaching it too late.

A preliminary experiment showed that to investigate effects of size of fish, photoperiod regimes, and smoltification in juvenile salmon, the size of fish needed at the start of the experiment in September should be about 8 cm. The intention of this study was to test the hypothesis that there is a threshold length important for the segregation of length–frequency into two modes. The experiments were designed to use groups graded into lengths near this "threshold" to study the effect of different temperature and photoperiod regimes on the development of bimodality.

Materials and Methods

Fish and Preexperimental Conditions

The experiments were carried out at the commercial fish farm Salar A/S in Ålvik, Norway, where heated effluent from an industrial plant was mixed with water of ambient temperature to provide the desired experimental temperatures. The stock at the farm is a mix of different Norwegian river populations. It has been held in culture for at least 10 yr. Individuals that grow fast and mature after at least 2 yr in the sea have consequently been chosen as brood fish. Eggs were hatched throughout the spring and family groups were not separated. The fish, kept in 5-m-diameter fiberglass tanks from first feeding until September at temperatures between 8 and 14°C, were regularly treated with malachite green to prevent fungus. Light was provided by neon tubes about 3 m above tanks giving an intensity of 24 lx at the water surface and 8 lx at the bottom of the tank and was continuous at least through the first summer. Daylight reaching the tanks from windows was less than 0.1 lx, measured on a rainy day in the beginning of February. Feeding regimes (7-min intervals) were continuous (24 h/d). Continuous feeding has been applied at this hatchery for several years because of positive practical experiences, including high growth rates and small problems with eye biting.

Experimental Design

The fish were first fed in May. The mean length was 81.4 mm on December 13, with a unimodal length–frequency distribution. To establish a group of approximately threshold size, the largest fish were removed by grading from December 13 to 15. The new group of 1920 individuals was held in a 1.7-m^3 tank under continuous light and high temperature (usually 10–11°C, range 2–15°C; Fig. 1). Fish for the experiment were chosen from this experimental stock.

The experimental design consisted of narrow length groups that were given different environmental conditions. The length groups were produced by selecting fish of a total length of 70–80 mm from the basic group and randomly dividing them into further treatment groups. This procedure was conducted twice, first on December 14–15 (Experiment A) and again on January 26 (Experiment B). Since Experiment B was a replication of Experiment A, the fish were combined in the same nine tanks, the new group being identified by fin-clips of adipose and pelvic fins. Fin-clips also separated the smaller (70–75 mm) from the larger fish (76–80 mm) in Experiment B.

Light, temperature and hours of feeding were varied to provide nine treatments (Table 1) in nine 1.5-m-diameter fiberglass tanks containing about 1.1 m^3 of water (water depth 0.6 m) placed in one row in an annex to the hatchery. The roof and one long side wall were translucent so that the tanks with constant light regimes were covered and provided with 60-W light bulbs giving light intensities at the water surface of 236 lx just beneath the light bulb, 190 lx near the wall of the tank, and 71 lx at the bottom. On a rainy day in February, the natural light intensity in one of the uncovered tanks was 165 lx at the water surface and 85 lx at the bottom. "Low" and "high" temperature regimes were used (Fig. 1). The different treatments and size classifications gave a total of 27 identifiable groups, 9 in Experiment A and 18 in Experiment B. There were lower total numbers in Experiment B than in Experiment A (Table 1) because of a shortage of fish in the desired size interval. The groups were measured (total length) at regular intervals about once a month until June 13 when smolts and smoltifying fish from the high-temperature groups, based on their appearance, were culled. The remaining fish (lower mode) and all fish in the treatment groups at the lowest temperature (A2, B2, A8, A9, B8, and B9) were also measured on July 11 and August 23, when the experiments ended and the fish were examined for precocious maturation. Seawater of about 0.5 ppt salinity was added to increase and stabilize pH. The fish were fed in surplus using a mix of Tess Elite 2.0 granulat and 1.8-mm pellet at 7-min intervals.

When morphological smoltification was observed in a group in spring and summer, fish were graded on the basis of appearance into five categories from 1 (smolt) to 5 (parr-like), in accordance with the schemes of Johnston and Saunders (1981) and Gorbman et al. (1982). Here the grading is simplified to smolt (1), smolt-like (2), and transitional (3, 4, and 5). The parr marks of transitional fish are visible, but to different degrees, being partly obscured by the silvery appearance of the scales. These fish may also be yellowish with weakened parr marks, and they do not have intense black pigment at outer edges of the pectoral and caudal fins as do the smolt-like fish. The smolt-like fish are slender in appearance and the back is darker than in transitional fish. Smolt-like fish are silvery in appearance but may still have a yellowish glimmer, absent in smolts, under the lateral line and posterior part of the head.

Because of leaks in some tanks, six of the groups were reduced in number or lost. The mortality in the groups at natural light and low temperature (A2 and large fin-clipped fish in B2) was size specific (Table 2), and therefore, the unbiased mean sizes of these groups at the start of the experiments were estimated. Surviving and dead fish were ranked according to their lengths in February. The lengths of the fish at the start of the experiments were also ranked, and fish of corresponding ranks to the dead fish were subtracted before the new means were estimated. The mean start length for A2 was increased from 76.2 to 77.7 mm, while that of the largest fin-clipped group of B2 was increased from 78.3 to 78.5 mm. The estimated bias for the smallest group of B2 was less than 1 mm.

Results

Under continuous light and equal temperature conditions, there were no differences in growth between the two feeding regimes, continuous feeding (groups B3, A8, and B8) and feed-

TABLE 1. Treatment groups in Experiments A and B. For each treatment group in Experiment A and its replicate in Experiment B, asterisks indicate the temperature and photoperiod given. Photoperiods were continuous (24) or natural (N). 2W, 4W, and 6W indicate fish held at low temperature (see Fig. 1) for the first 2, 4, or 6 wk, respectively. Feeding followed light regime (there were four exceptions, see below). Numbers of individuals in Experiment A and small (<76 mm) and large (>75 mm) fin-clipped groups in Experiment B are also given.

Group		Photoperiod		Temperature					No. of fish		
15/12	26/1[a]	24	N	Low	6W	4W	2W	High	A	B < 76	B > 75 mm
A1	B1		*					*	80	39	41
A2	B2		*	*					82	31	37
A3[b]	B3	*						*	83	34	36
A4[c]	B4[c]	*						*	81	36	44
A5	B5	*					*[d]		80	28	30
A6	B6	*				*[d]			80	27	32
A7	B7	*			*[d]				84	23	32
A8	B8	*		*					80	37	33
A9[c]	B9[c]	*		*					80	37	44

[a]Starting day and month.
[b]A3 was returned to the basic group in error and was restarted on January 29.
[c]Feeding followed natural photoperiod.
[d]Eight hours of light and feeding each day when held at low temperature.

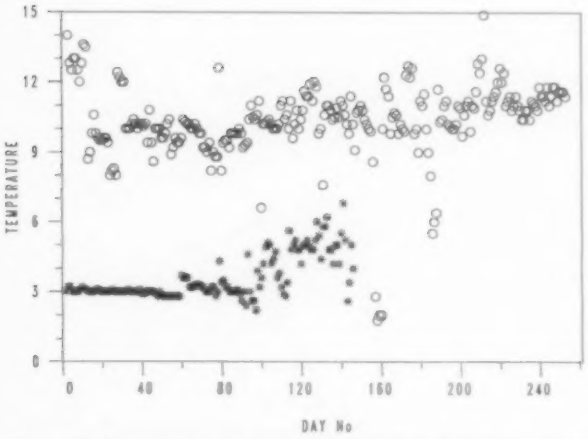

FIG. 1. Daily temperatures from December 13 (day 1) to August 23 where asterisks represent low temperature and circles represent high temperature. All fish were held at high temperature after day 145.

TABLE 2. Cases of high mortality. If possible, a *t*-test was performed to test if mortality was size specific. Large and small refers to fin-clipping.

Group	Date	Mortality (%)	Size difference (mm)	Significance (α)
A4	9.2	49	0.6[a]	
B4 large	9.2	44	2.0[a]	
B4 small	9.2	53	0.8[a]	
A2	19.2	32	−3.8[b]	<0.001
B2 large	19.2	40	−2.0[b]	<0.02
B2 small	19.2	43	−1.0[b]	NS
A7	10–25.5	100		
B3	10–25.5	100		

[a]Mean length of the dead fish minus the average size of the group on January 26.
[b]Mean length of the dead fish minus the length of the survivors on February 23.

ing according to photoperiod (B4, A9, and B9). The length of the parallel groups (B3 versus B4, A8 versus A9, and B8 versus B9) were compared at the different dates of measurements: fin-clipped length groups were compared separately. Although there was a temporary difference between the largest fin-clipped groups in B3 and B4 on March 3 (*t*-test, $P < 0.02$), statistical differences were not found on other dates. The two other groups sharing light and temperatures regimes but not feeding regimes (A3 and A4) could not be compared because A3 was started 2 wk later than A4 (Table 1).

Photoperiod, not the temperature regimes, influenced the degree of bimodal segregation (Fig. 2). All groups on natural light developed a clear bimodal length–frequency distribution (Fig. 2). Under continuous light the bimodality was absent or weak; the distributions became skewed to the left rather than bimodal (Fig. 2). One cause for these differences between the two light regimes was consistent differences in the growth patterns during the experiment. The development of bimodality was initiated at sizes below 10 cm under natural photoperiod, where the fish rapidly segregated into slowly and rapidly growing fractions, regardless of temperature (Fig. 3). This clear pattern, i.e. the gradual differentiation of the two size groups during the presmolt growth phase, was not seen in any of the groups given continuous light where the fish continued to enter the length interval between the modes (Fig. 4).

The bimodality developed according to the length of the fish at transfer, and not the time of transfer to natural photoperiod. The probability of belonging to the upper mode the next spring (or summer) increased sharply with the length of the fish at the start of the experiments (Fig. 5). The fish smaller or longer than a threshold length of about 75 mm generally developed into lower or upper mode fish, respectively. The independence between the time of transfer and the bimodality is seen from the similarity between the replicates (Fig. 2–4). The fish of 70–80 mm that were transferred to natural light in December were larger at that time than the fish that reached 70–80 mm and were transferred in January (Table 3). Approximately 45% of the fish in Experiment B were smaller than 70 mm in December (this can be estimated from the percentages presented in

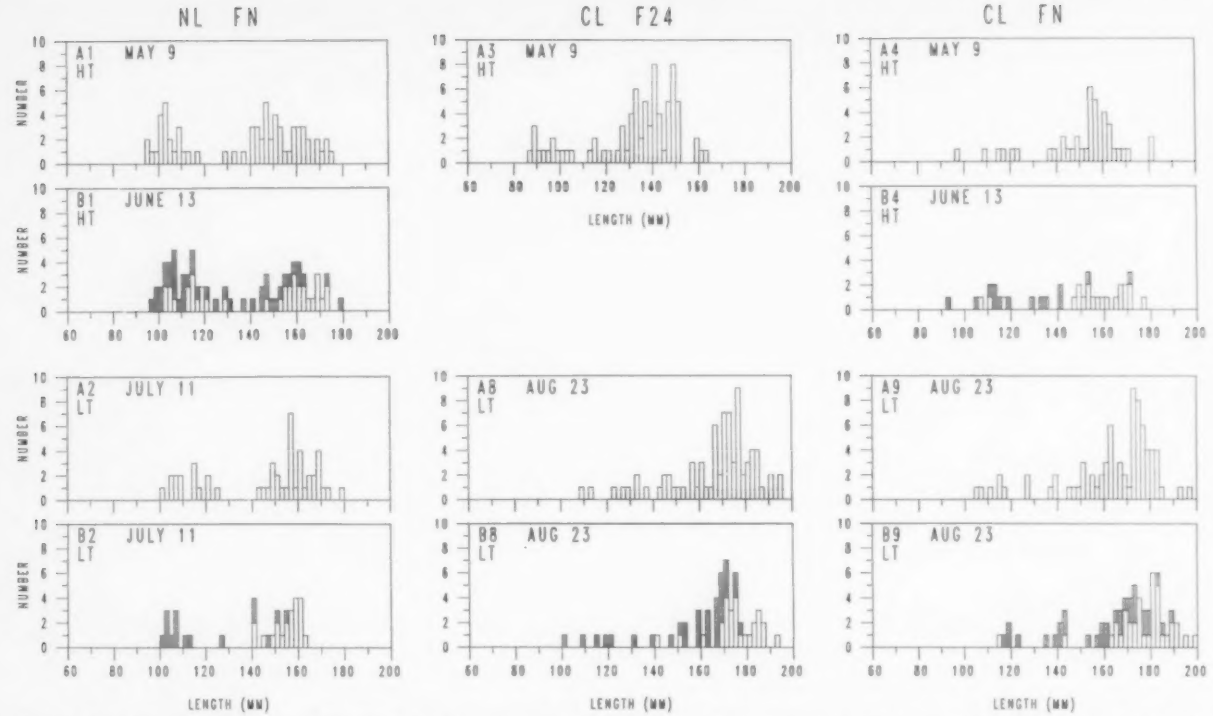

FIG. 2. Length distributions late or at the end of the experiment (dates in panels) of groups reared under natural (NL) or continuous light (CL) and fed continuously (F24) or according to natural photoperiod (FN). HT and LT are high and low temperature (Fig. 1). Shaded (small fin-clipped fish) and white bars (large fin-clipped fish) are stacked. A or B followed by a number refers to the photoperiod and temperature treatment of groups of fish in Experiment A (starting December 14–15) and Experiment B (starting January 26) (see Table 1).

Table 3 because all fish of 70–80 mm were removed in January).

Temperature drops for 2, 4, or 6 wk combined with constant 8 h of daily light did not trigger the development of bimodality (Fig. 6).

The smolt classifications of the fish were evidently influenced by the light regime (Fig. 7). Under natural light the upper mode fish rapidly changed from parr to smolt appearance during spring. The morphological smoltification under continuous light was rare or incomplete for fish of "smolt size."

The light regimes influenced both the occurrence and gonadal development of precociously mature males. There were higher percentages of precociously mature males under natural light, but the maturation was advanced under continuous light (Fig. 8). Precociously mature males were only observed among the lower mode fish under natural light and among the smallest fish under continuous light.

Discussion

The use of graded length groups in the present work was an attempt to find a simple and informative way to study the development of bimodality. Grading may lead to loss of information, but it was confirmed that small size differences can have a pronounced effect on the development of length–frequency bimodality. The fin-clipping may have influenced the results. Nicola and Cordone (1973) concluded that this marking method had dramatic effects on survival of rainbow trout (*Oncorhynchus mykiss*, formerly *Salmo gairdneri*) in a natural environment, but significant differences in growth between marked and unmarked fish were not detected. Nor did Holm and Fernø (1983) find significant differences in growth between fin-clipped and unmarked juveniles of Atlantic salmon, but they observed that fish lacking pelvic fins were the least aggressive and least often the target of aggressive acts.

Light quality differed between natural and artificial light in the present experiments. However, Stefansson and Hansen (1989) found no correlation between spectral composition of light and growth rate or smoltification.

Despite these deficiencies, the experiments showed that the establishment of bimodal segregation of length–frequency distribution was clearly dependent on (a) initial length and (b) natural photoperiod. The experiments also showed that decrease in temperature was not a trigger for this mechanism.

Threshold Length

Elson (1957) was the first to propose that smoltification in the spring was linked to the length of the fish. He suggested that presumptive smolts had to reach a length of about 10 cm during the autumn before smoltification. The idea of a critical minimum size has been accepted as one factor in the development of a bimodal length–frequency distribution, but there are varying opinions on its importance. Thorpe et al. (1980) proposed that the upper mode fish must reach a minimum size in the autumn, but they believed that the decision to become an upper mode fish had already been taken in mid- or late summer. This view implied that bimodality is a consequence of

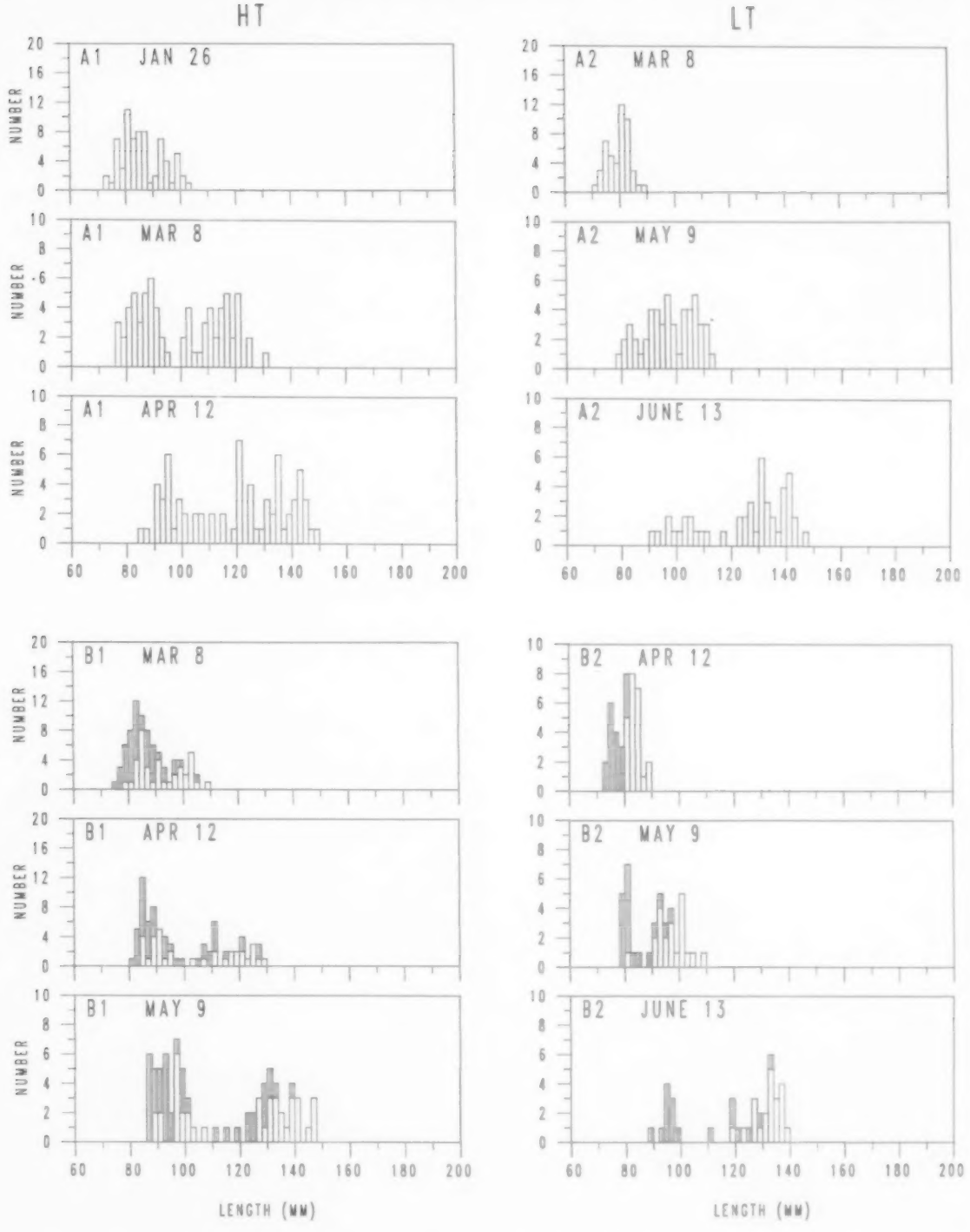

FIG. 3. Length–frequency distributions at three different times for groups under natural light and high (HT) or low (LT) temperature. Shaded (small fin-clipped fish) and white bars (large fin-clipped fish) are stacked.

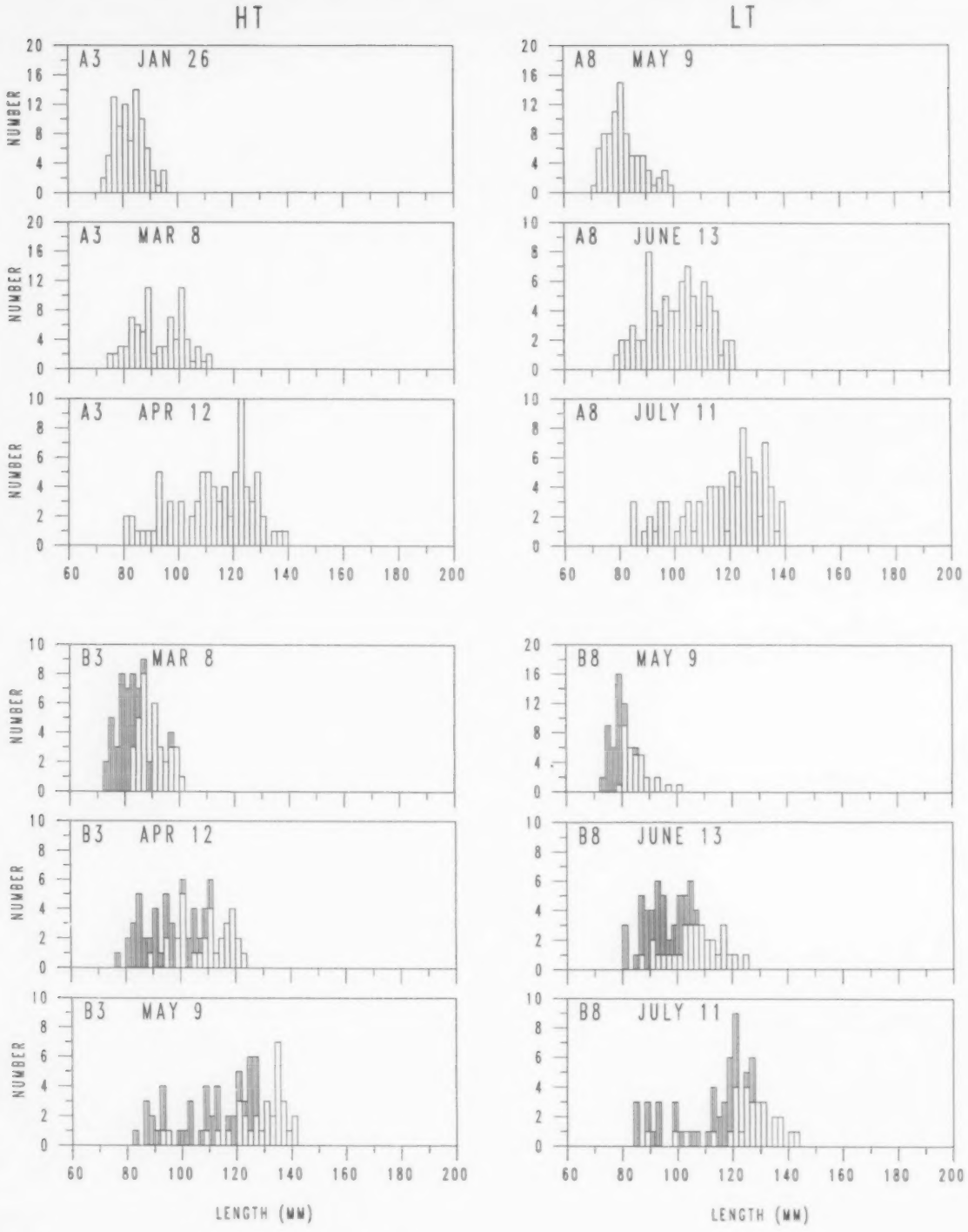

FIG. 4. Length–frequency distributions at three different times for groups under continuous light and high (HT) or low (LT) temperature. Shaded (small fin-clipped fish) and white bars (large fin-clipped fish) are stacked.

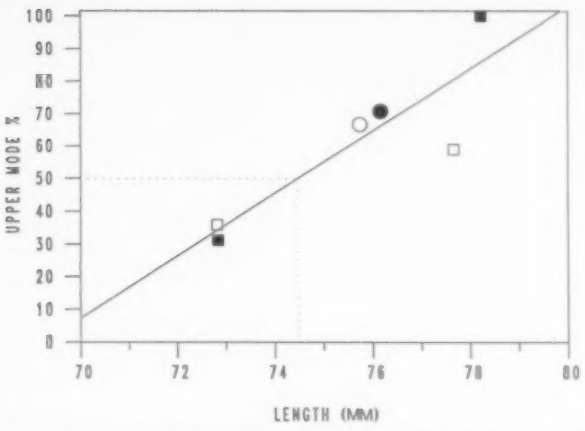

FIG. 5. Mean length at the start of the experiments of the groups from Experiment A (circles) and Experiment B (squares) that were held under natural light and low (solid symbols) or high (open symbols) temperature versus the proportion of fish in the upper mode in spring. The linear regression line is included. Dotted lines mark the theoretical threshold length from which 50% of the fish enter the upper mode. Regression statistics: $\alpha < 0.05$, $R^2 = 0.77$. (If the mean lengths of A2 and B2 are adjusted for mortality, then $\alpha < 0.05$ and $R^2 = 0.79$ and the length corresponding to 50% upper mode fish increases from 74.5 to 74.7 mm.)

TABLE 3. Mean size of the experimental stock after the start of Experiment A and before the initiation of Experiment B. Percentages of fish below 70 mm, between 70 and 80 mm, and above 80 mm are also given.

Date	Mean length (mm)	Sample size	<70 mm (%)	70–80 mm (%)	>80 mm (%)
December 17	73.0	165	35	43	22
January 26	80.9	143	20	33	47

events that take place earlier, before the fish reaches the threshold length. Kristinsson et al. (1985) presented evidence that fish enter a faster growing phase above a critical length. Their hypothesis, that the control of growth occurs a few days before the increased growth rate, supports that of Skilbrei (1988) who suggested that attainment of theshold length is the stimulus triggering increased growth rate.

In the present study, the strong similarity in results between Experiments A and B is of particular interest. The fish in Experiment B were smaller than those in Experiment A when Experiment A was started. Nevertheless, when fish in Experiment B were transferred to natural light 6 wk later at corresponding sizes, they segregated into similar porportions of lower and upper mode fish, implying that the length of the juvenile at transfer was much more important than the time of transfer. This replication of results strongly supports the idea of a critical threshold length and would seem to contrast with the concept that the physiological decision was taken the previous summer.

Threshold length may be defined as the initial length at which 50% of the fish enter the upper mode, although threshold range would be a more meaningful expression in a biological sense. The threshold length was about 75 mm (total length) for the Norwegian fish examined here, but it may be slightly higher if it takes some time for the fish to respond to the new photoperiod. It has been suggested that the threshold length (Skilbrei 1988), or critical size (Thorpe et al. 1980), may vary between different populations of Atlantic salmon. Kristinsson et al. (1985) found a threshold length to range from 80 to 120 mm (fork length) in Canadian Atlantic salmon. From the data presented by Metcalfe et al. (1988), there is reason to believe that the critical threshold level is close to 70 mm (fork length) for Scottish salmon.

Increased growth rate of upper mode fish and reduced growth rate of the individuals in the lower mode (Higgins 1985) are the two primary causes of bimodality in the length–frequency distributions. In the present study, the use of narrow length intervals ensured that the growth rates of both potentially lower and upper mode fish were almost equal before the start of the experiments. The lower growth rate of fish below the threshold length may have been a result of reduced appetite, which has been demonstrated for lower mode fish by Metcalfe et al. (1988) in an experiment under simulated natural photoperiod. These authors suggested that the lower mode fish maximize their survival chances by remaining almost dormant over the winter, when costs of foraging are high.

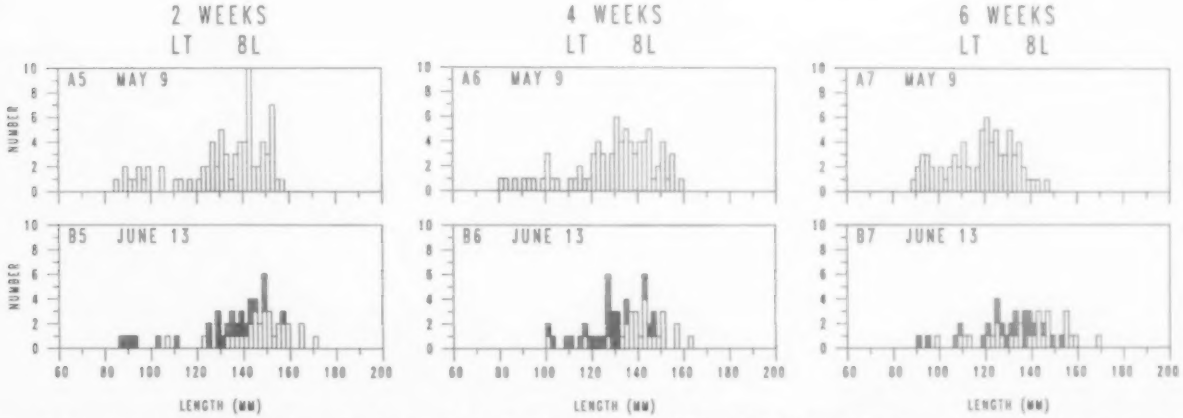

FIG. 6. Length–frequency distributions after 2, 4, or 6 wk of cold water (LT) and 8 h of light (8L) and feeding and thereafter high temperature and continuous light. Shaded bars (small fin-clipped fish) and unshaded bars (large fin-clipped fish) are stacked.

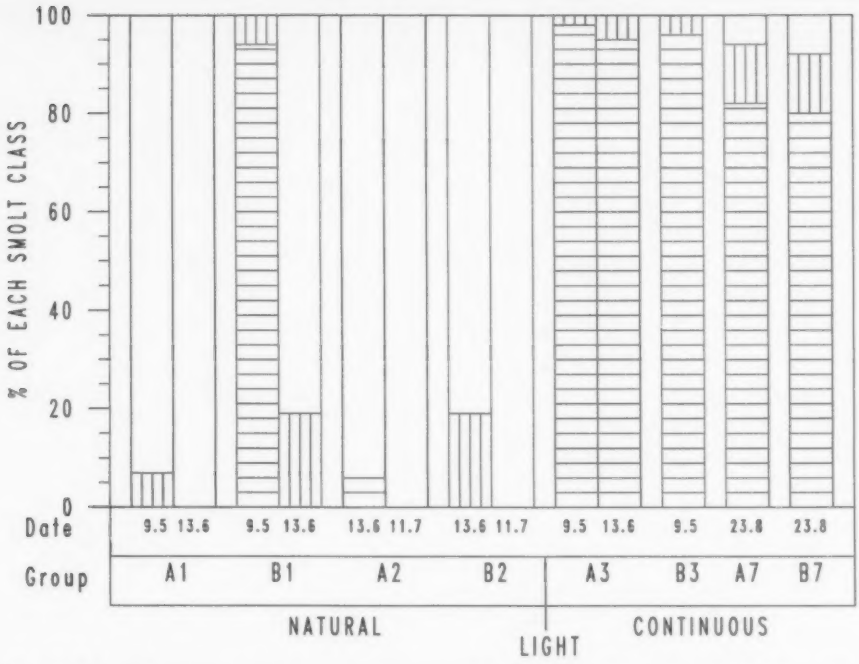

FIG. 7. Bar graphs representing the smolt appearance of the fish. In each group (A and B followed by a number, see Table 1), the percentage of the upper mode fish (under natural light) or fish above 12 cm (under continuous light) belonging to the classifications "transitional" (horizontal shadings), "smolt-like" (vertical shadings) or "smolt" (white) is given for various dates.

Light Stimulus

The individual decision to grow slowly or rapidly was determined by the threshold length when natural photoperiod was introduced in December or January. In the following summer, there was also a small number of fish of "lower mode size" under the continuous light regime, possibly because of an endogenous component in control of growth. Parr appear to show an annual cycle in growth patterns when held under constant environmental conditions (Eriksson and Lundqvist 1982), but it can be concluded that bimodality developed in response to natural photoperiod in the present experiments. However, it is not clear whether the critical stimulus was the minimum daylength or the daily rate of change. It is possible that the response to a daily rate of change is dependent on the time of year. Bimodal length–frequency distributions of Norwegian (Skilbrei 1988), Scottish (Thorpe 1977), and Canadian (Bailey et al. 1980) salmon usually develop during autumn, under decreasing daylength, and not under increasing daylength as in the present study. The time of introduction to natural photoperiod may not be critical, at least between autumn and winter months.

The lack of bimodal development in the groups reared under 8 h of light for 6 wk does not support the hypothesis of a minimum daylength as the key stimulus. However, 6 wk is probably too short a time for bimodality to develop at low temperature, since Saunders et al. (1987) found a much more developed bimodality when juveniles were given 16 h daylength for 15 wk compared with continuous light.

The theory that the interface between threshold length, photoperiod, and the time of year determines the bimodal segregation may at first seem to disagree with the following two reports. Clarke and Shelbourn (1986) reported that the bimodal growth of coho salmon (*Oncorhynchus kisutch*) was probably a function of the photoperiod at the time of first feeding. Villarreal et al. (1988) suggested that growth is maintained if the feeding opportunities, influenced by photoperiod, are above a certain threshold level 2–3 mo after first feeding. The explanation may be that the duration of the period from first feeding to the time of the "critical" light stimulus and the conditions for growth are different under the different light regimes in these three studies. Both changes in the critical time and the conditions for growth will influence the proportion of fish below the threshold length. Also, the efficiency of the winter light stimulus may differ between different light regimes.

Upper mode fish grew faster under natural than under continuous light. This may have resulted from differences in light quality because the reduction in daylength from artificial continuous light to natural light and daylength should have given the opposite effect. The explanation may also be an effect of the subsequent increasing daylength, which has been shown to stimulate feeding, appetite, and feed conversion (Saunders and Henderson 1970; Komourdjian et al. 1976), i.e. that photoperiod is acting as a synchronizer of an endogenous rhythm of appetite and growth in salmonids (Villarreal et al 1988; Clarke et al. 1978).

Temperature, Not a Seasonal Cue?

Sharp reductions in temperature for shorter or longer periods did not lead to a clear bimodal length–frequency distribution. It is therefore likely that juveniles above 70 mm do not use variations in temperature as a seasonal cue to determine whether

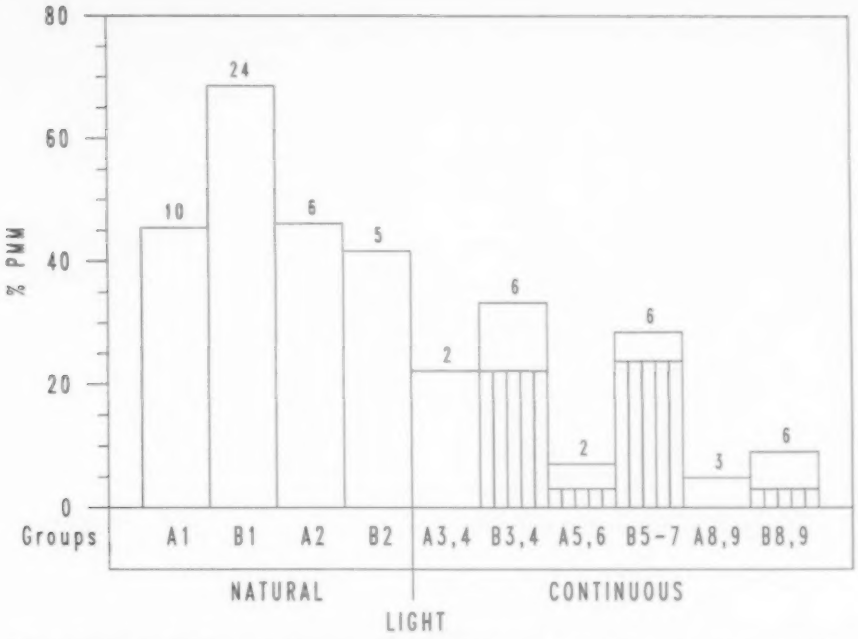

FIG. 8. Percentages of precociously mature males (PMM) in each group on August 23, separated into maturing (white bars) or ripe males (shaded bars). In maturing individuals, the length of the gonads was usually 70–90% of the body cavity under natural light; in a few individuals, it was about 50%. In the groups A8,9 and B8,9, where the largest fish of smolt size were not sorted out in June, the percentages are calculated from fish of "lower mode size." The upper size boundary was set to 170 cm after comparison with the lower mode fish under natural light. The sum of the numbers of mature or maturing individuals is given at the top of each bar.

or not to smoltify the next spring. This observation is in agreement with the findings of Clarke et al. (1978) for underyearling sockeye (*Oncorhynchus nerka*) and coho salmon. The assumption of Simpson and Thorpe (1976) that falling temperature in autumn causes bimodality is not supported by the present study. However, the temperature, because of its general influence on growth and metabolism (Brett et al. 1969; Iwama and Tautz 1981), will limit the number of fish that can reach the threshold length before the shortening of daylength. For juveniles of Atlantic salmon, both smaller and larger than 7.5 cm, there is a close relationship between temperature and growth rate (Austreng et al. 1987).

Precocious Maturation

The higher incidence of precocious maturation under natural compared with continuous light may be related to length and photoperiod or only to photoperiod. From Canadian experiments, Bailey et al. (1980) suggested that a threshold size must be reached before maturation can occur. These authors found precociously mature males in both size modes and they concluded that the size threshold for precocious maturation must be lower than that for smolting the following spring. In Scottish experiments (Thorpe and Morgan 1980), and in the present study, there were precociously mature males only in the lower mode of the length distributions. In these two populations, it still cannot be concluded that early maturation and smolting are dependent on the same threshold length. The decision to mature as 1+ may have been made after the decision not to smoltify as 1-yr smolts. If the decision to mature must be made early in the growing season to be synchronous with the maturation of larger migrating salmon, as proposed by Bailey et al. (1980), then the lower incidence and the early ripening in August of precociously mature males under continuous light may result from the lack of proper environmental cues.

Practical Implications

Culture of salmon makes a priority of improving the numbers of 1-yr smolts and the smolt quality. The first target may be reached if the parr are not transferred to natural light in the autumn before they exceed the threshold length. One may use heated water, extra feeding, and artificial lengthened daylight during the first part of the freshwater phase to ensure that the parr surpass the threshold for becoming smolts the next spring. The temperature requirements are lowered when the parr have been transferred to natural light. According to the present study, the smolt size and the time of smoltification will be influenced, not the numbers of smolts if they were above the threshold length before the shortening of daylength. One should *not* use continuous light during the winter and spring even though the number of fish of upper mode size may be higher under this light regime. Continuous light applied throughout the rearing period interfered with the morphological smoltification in the present study. It also lowers the salinity tolerance (McCormick et al. 1987) and reduces the subsequent postsmolt growth in

seawater (Saunders et al. 1985). The fish farmer should therefore decrease daylength in late autumn so that the daylength can be increased during late winter and spring. Proper smoltification involves a number of physiological events (Hoar 1976; Folmar and Dickhoff 1980) that are synchronized under increasing daylength (Saunders and Henderson 1970; Komourdjian et al. 1976; Saunders et al. 1985; McCormick et al. 1987). The relationships between body length, bimodality, and photoperiod may be utilized to maximize the production of 1-yr smolts. We must, however, keep in mind that the threshold length, and possibly the growth response of the parr to changing daylengths, may vary between stocks. Hatchery production schemes should therefore be preceded by experiments to characterize the growth patterns of the specific strains of Atlantic salmon.

Acknowledgements

I thank H. Waagebø Jenssen and the staff at Salar A/S (Ålvik, Norway) for practical assistance and care of the fish and G. Bakke for assistance during collection and preparation of the material. I am grateful to K. Pittman, A. Fernø, Ø. Skåla, G. Nævdal, J. C. Holm, G. Johnsen, T. N. Pedersen, and H. Sægrov for reading the manuscript. The study was financially supported by BP Development Ltd, Norway. There were also contributions from the Division of Aquaculture (Institute of Marin Research), the Department of Fisheries Biology (University of Bergen), and the Norwegian Fisheries Research Council.

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Selecting Loci for Genetic Stock Identification Using Maximum Likelihood, and the Connection with Curvature Methods

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Millar, R. B. 1991. Selecting loci for genetic stock identification using maximum likelihood, and the connection with curvature methods. Can. J. Fish. Aquat. Sci. 48: 2173–2179.

Maximum likelihood theory is used to predict the precision of genetic stock identification composition estimators — prior to collection of the mixed fishery sample. It is shown how this allows the researcher to plan the genetic stock identification study, through specification of sample size and choice of genetic data to assay, so as to maximize estimator precision. The curvature methodology used inGomulkiewicz et al. (1990. Can. J. Fish. Aquat. Sci. 47: 611–619) is shown to be closely related to the maximum likelihood approach. In that study, interpretation of results is complicated by the use of an overparametrized curvature measure. Here it is shown that when applied to an appropriately parametrized likelihood function the curvature methodology reproduces the maximum likelihood theory.

On a utilisé la théorie du maximum de vraisemblance pour prévoir la précision des estimateurs de composition pour l'identification des stocks génétiques avant la cueillette d'un échantillon de pêche mixte. On montre comment elle permet au chercheur de planifier l'étude d'identification du stock génétique à partir de la taille de l'échantillon et du choix de données génétiques qui permettent de maximiser la précision de l'estimateur. La méthode de la courbure utilisée par Gomulkiewicz et al. (1990. J. can. sci. halieut. aquat. 47 : 611–619) se révèle très proche de la méthode du maximum de vraisemblance. Dans cet étude, l'interprétation des résultats est compliquée par l'utilisation d'une mesure de la courbure sur-paramétrisée. On montre ici que lorsqu'on l'applique à une fonction de vraisemblance bien paramétrisée, la méthode de la courbure reproduit la théorie du maximum de vraisemblance.

Received November 22, 1990
Accepted May 28, 1991
(JA814)

Reçu le 22 novembre 1990
Accepté le 28 mai 1991

Precision of stock composition estimators obtained by genetic stock identification (GSI) depends on the amount of statistical information yielded by a sample taken from the mixed stock fishery. This paper formalizes the above assertion and shows that the expected information content of a mixed fishery sample is dependent on the mixed fishery composition, the genetic data being measured, and the size of that mixed fishery sample. Since the researcher using GSI has some control over the last two of these factors, knowledge of their influence on the expected information content of the mixed sample can be used to design the composition estimation project. That is, prior to collection of the mixed fishery sample, the researcher can choose which loci to assay and can specify the size of the mixed fishery sample necessary to attain a desired level of composition estimator precision. Similarly, subject to budgetary and logistical constraints, these choices can be made to maximize estimator precision.

The choice of loci to assay typically takes the form of selecting a subset of loci from those in the collection of baseline data — a collection of allele (or genotype) frequencies observed from samples of known origin fish. An example of such a collection is given in Marshall et al. (1990). It will be assumed in this study that the samples of known origin fish are sufficiently large that these frequencies are representative of the true frequencies.

Here it is assumed that the GSI estimator of composition is obtained by maximum likelihood. Given the subset of loci to be used, it is the composition which results in the combination of baseline genotype frequencies that is most likely to give rise to the genotype frequencies observed in the mixed fishery sample. Extensive detail is provided by Milner (1983), Fournier et al. (1984), Millar (1987), Wood et al. (1987), and Millar (1990), and the log-likelihood equation for the mixed fishery sample is given in the Appendix by equations (A.1) or (A.2).

In the Theory section it is seen that the matrix of second derivatives (the Hessian matrix) of the log-likelihood function is fundamental in estimating the precision of maximum likelihood estimators. Millar (1986, unpublished report for Washington State Department of Fisheries, Olympia, WA 98504) implemented in FORTRAN a method of selecting loci for GSI studies by evaluating the diagonal elements of the Hessian matrix. Since the log-likelihood function depends on the observed sample from the mixed fishery, Millar enabled evaluation of the Hessian (prior to collection of the mixed fishery sample) by using the expected mixed fishery sample from a mixed fishery with equal contribution from every stock. This implementation was restricted to selecting loci from the baseline collection and it did not attempt to quantify the expected precision of resulting stock composition estimates.

Gomulkiewicz, Brodziak, and Mangel (1990) (henceforth abbreviated to GBM) motivated a more complex study by using curvature arguments and showed that curvature is a function of the Hessian matrix. The theoretical basis for their approach is sound — indeed, this geometrical motivation should have exactly reproduced the maximum likelihood results summarized in the Theory section below. However, in implementing the curvature argument it was not taken into account that the

sum of the composition estimates (estimated mixing proportions) is unity and an overparametrized Hessian was calculated. Thus, the curvature measure calculated does not correspond to the curvature measure that is motivated in the earlier portion of their paper (see Fig. 1 and 2 of GBM). This resulted in a measure of loci "utility" that is of limited value in choosing the best GSI design.

Here the appropriately parametrized Hessian is used and moreover, by following a statistical development the resulting measures of loci performance have immediate and direct quantitative application to composition estimator performance. The Numerical Results section presents these performance measures for the same diallelic loci used in table 3 of GBM, giving the average and worst performance of these loci over a uniform grid of possible three-stock mixture compositions. In addition, a table giving the percentage of times (over the grid) that each locus performed better than each of the other loci is given. Of particular interest is the case that locus A performs better than locus B for all possible mixed fishery compositions, whereupon we shall say that locus A dominates locus B.

If prior knowledge of the unknown mixed fishery composition is available, then the performance of loci at fixed values of composition or over a restricted range should be used rather than an average over all possible compositions. An example is presented in the Numerical Results section and simulations verify that the loci performance measure accurately determines the precision of the composition estimates, prior to mixed fishery sampling. It is seen that use of the overparametrized Hessian matrix can give very different results.

Theory

Consider a mixed stock fishery composed of S stocks and denote its composition vector by $\Theta = (\theta_1,\theta_2,\ldots,\theta_S)$. The composition proportions, θ_j, $j = 1,\ldots,S$, are the parameters over which the likelihood function for the mixed fishery sample is maximized and the maximizing composition is denoted by $\hat{\Theta} = (\hat{\theta}_1,\hat{\theta}_2,\ldots,\hat{\theta}_S)$. Since they are proportions the sum of these parameters is unity; therefore it is enough to know the value of just $\theta_1,\theta_2,\ldots,\theta_{S-1}$. In statistical terminology one would say that there are $S-1$ free parameters since the Sth parameter is determined by the others. These free parameters will be denoted by $\Theta^* = (\theta_1,\theta_2,\ldots,\theta_{S-1})$ and their estimated values by $\hat{\Theta}^* = (\hat{\theta}_1,\hat{\theta}_2,\ldots,\hat{\theta}_{S-1})$. (Note: The choice of free parameters is not unique and could be any $S-1$ of the S parameters. The inferences made are in no way affected by the particular choice.)

The steps presented below describe a procedure for calculation of the asymptotic covariance matrix of maximum likelihood estimators. To emphasize the generality of this methodology the model parameters are denoted by Λ, and their maximum likelihood estimates by $\hat{\Lambda}$. The theory of maximum likelihood depends on certain "regularity" conditions being satisfied. One such condition is that the asymptotic covariance matrix, Σ_Λ, must be derived using free parameters (Wilks 1962; Lehmann 1983). (This is usually stated as the requirement that Λ be an element of an open subset of the parameter space.) If the regularity conditions are satisfied, then Σ_Λ is given by the following steps.

Step 1: Calculate $H(\Lambda)$, the Hessian matrix of second derivatives (with respect to Λ) of the log-likelihood function.

Step 2: Calculate $I(\Lambda) = -E(H(\Lambda))$, where E denotes expectation assuming sample size 1 and true parameters Λ.

Step 3: Calculate $\Sigma_\Lambda = \dfrac{I^{-1}(\Lambda)}{N}$ where $I^{-1}(\Lambda)$ is the inverse of $I(\Lambda)$ and N is the size of the (random) sample.

Remark 1: As an alternative to steps 1 and 2 above, $I(\Lambda)$ is also given by the covariance matrix of the first derivatives of the log-likelihood (Lehmann 1983, p. 126).

Remark 2: A variant of step 2 is given by evaluating $H(\Lambda)$ at the data rather than taking its expectation. There does not appear to be a consensus on which form of step 2 is preferred (e.g. Efron and Hinkley 1978, which includes comments from several discussants) and computational convenience may dictate which to use in practice. In this study the expectation of $H(\Lambda)$ is evaluated because measures of predicted composition estimator precision are required prior to obtaining data (the mixed fishery sample).

The covariance matrix Σ_Λ is "asymptotic" because the theory behind it is based heavily on the Central Limit Theoreom (CLT). That is, Σ_Λ is an approximation to the true covariance matrix of $\hat{\Lambda}$ and the degree of approximation improves as the sample size increases (since the CLT assumes large sample size). However, in some special cases (e.g. maximum likelihood estimation of the mean of a Normal distribution (with known variance) or Binomial distribution), Σ_Λ is exact, regardless of sample size. Since Λ is unknown, an estimate of Σ_Λ is given by using the maximum likelihood estimates $\hat{\Lambda}$ in steps 2 and 3 above.

In the statistical literature, $I(\Lambda)$ is called the information matrix and $NI(\Lambda)$ gives the amount of statistical information contained in the sample. Note that Σ_Λ is the inverse of $NI(\Lambda)$ and that the precision of the estimates increases (i.e. the values in Σ_Λ decrease) as statistical information increases. In particular, Σ_Λ is inversely proportional to sample size and therefore the standard deviations of the elements of $\hat{\Lambda}$ are inversely proportional to the square root of sample size.

In the mixed stock context, $\Lambda = \Theta^*$, so $H(\Theta^*)$ is an $(S-1)\times(S-1)$ matrix of second derivatives and Σ_{Θ^*} is the $(S-1)\times(S-1)$ asymptotic covariance matrix for Θ^*. Since $\hat{\theta}_S = 1-\hat{\theta}_1-\hat{\theta}_2-,\ldots,-\hat{\theta}_{S-1}$ the asymptotic covariance matrix of $\hat{\Theta}$, Σ_Θ, is derived from Σ_{Θ^*} using a standard statistical formula (equation (A.4), Appendix).

The implementation of GBM was motivated by considering the minimum curvature of the log-likelihood function as displayed in their fig. 1 and 2. These figures display the log-likelihood for a three-stock mixture as a function of the free parameters θ_1 and θ_2. The Appendix shows that an error in implementation occurred when it was claimed that the minimum curvature is proportional to the smallest eigenvalue of the $S\times S$ Hessian matrix. It is necessary to use the $S-1\times S-1$ Hessian matrix $H(\Lambda)$ (step 1) obtained from using free parameters. Calculation of $I(\Lambda)$ in step 2 removes the dependence on the mixed fishery sample and is equivalent to the approach motivated by GBM.

Example

The simplest case of a mixed fishery problem is one with two stocks and where the stock of origin of fish in the mixed sample can be determined without error. Although an unlikely scenario in practice, this can be considered a limiting case in GSI, since using several loci with large differences in allele

frequencies between the two stocks will come close to it. This example serves to demonstrate the concepts of the previous section and extending it to a general GSI study is conceptually no different, requiring only more algebra (see Appendix).

For ease of presentation, suppose that a diallelic locus is assayed for which only allele type A occurs in stock 1 and only allele type a occurs in stock 2. Since only two genotypes are possible (AA for stock 1 fish and aa for stock 2 fish), this example reduces to a binomial experiment. If $\Theta = (\theta_1, \theta_2)$ is the mixed fishery composition and N is the size of the mixed fishery sample, then the number of AA genotypes (i.e. stock 1 fish) observed in that sample, y, is distributed as a binomial (N, θ_1) random variable. The log-likelihood function for the mixed fishery sample, expressed as a function of the free parameter $\Theta^* = \theta_1$, is (to within a constant)

$$(1) \quad l(\theta_1) = y\log\theta_1 + (N-y)\log(1-\theta_1).$$

It is well known that the maximum likelihood solution to (1) is $\hat{\theta}_1 = y/N$ (Johnson and Kotz 1969).

To calculate the asymptotic variance of $\hat{\theta}_1$, we begin with step 1, evaluation of the Hessian, which in this case is a scalar rather than a matrix, since only one parameter is involved. The Hessian is

$$H(\theta_1) = \frac{-y}{\theta_1^2} - \frac{N-y}{(1-\theta_1)^2}.$$

Setting $N = 1$, replacing y by its expectation $E(y) = N\theta_1 = \theta_1$, and multiplying by -1 (step 2) gives

$$I(\theta_1) = -E(H(\theta_1)) = \frac{\theta_1}{\theta_1^2} + \frac{(1-\theta_1)}{(1-\theta_1)^2} = \frac{1}{\theta_1(1-\theta_1)}.$$

Step 3 gives the asymptotic variance of $\hat{\theta}_1$, for mixed sample size N, to be $\Sigma_{\theta_1} = I^{-1}(\theta_1)/N = \theta_1(1-\theta_1)/N$. The binomial is a special case in which the asymptotic variance is the true variance of $\hat{\theta}_1$ (Johnson and Kotz 1969). Since $\hat{\theta}_2 = 1 - \hat{\theta}_1$, the asymptotic covariance matrix of $\hat{\Theta} = (\hat{\theta}_1, \hat{\theta}_2)$ is (Seber 1977, p. 10)

$$(2) \quad \Sigma_\Theta = \frac{1}{N}\begin{pmatrix} \theta_1(1-\theta_1) & -\theta_1(1-\theta_1) \\ -\theta_1(1-\theta_1) & \theta_1(1-\theta_1) \end{pmatrix}.$$

Since θ_1 is unknown the variance of $\hat{\Theta}$ is estimated by replacing θ_1 with $\hat{\theta}_1$ in (2).

If one works with the overparametrized log-likelihood function

$$l(\Theta) = y\log\theta_1 + (N-y)\log\theta_2,$$

taking the second derivative of $l(\Theta)$ with respect to both θ_1 and θ_2 results in the Hessian matrix

$$\mathrm{H}(\Theta) = \begin{pmatrix} \frac{-y}{\theta_1^2} & 0 \\ 0 & \frac{-(N-y)}{\theta_2^2} \end{pmatrix}.$$

Setting $N = 1$ and taking the negative of the expectation of $H(\Theta)$ gives the (incorrect) information matrix

$$I(\Theta) = -E(H(\Theta)) = \begin{pmatrix} \frac{\theta_1}{\theta_1^2} & 0 \\ 0 & \frac{\theta_2}{\theta_2^2} \end{pmatrix} = \begin{pmatrix} \frac{1}{\theta_1} & 0 \\ 0 & \frac{1}{\theta_2} \end{pmatrix}.$$

Inverting this and dividing by actual sample size gives the (incorrect) asymptotic covariance matrix

$$\frac{1}{N}\begin{pmatrix} \theta_1 & 0 \\ 0 & \theta_2 \end{pmatrix}.$$

From the statistical perspective, GBM's numerical results are obtained from the incorrectly parametrized information matrix and therefore are of questionable value.

Measures of Loci Performance

For any given choice of assayed loci the expected precision of $\hat{\Theta} = (\hat{\theta}_1, \hat{\theta}_2, \ldots, \hat{\theta}_S)$ is given by the $S \times S$ asymptotic covariance matrix Σ_Θ (assuming sufficiently large sample size). To facilitate comparison between different choices of loci, it is helpful to summarize the covariance matrix by a single value. There are many possible summary values that could be used and the best one will depend on the preference of the researcher. The diagonal elements of Σ_Θ give the variances of the elements of the estimated composition vector $\hat{\Theta}$ and the sum of these variances could be used as the summary value. If one stock in the mixed fishery is of particular importance, then the variance of that one stock may be a better choice. With either of these summary values, the smaller it is, the better the performance of the assayed loci. Measures of performance for the chosen loci could therefore be given by inverting these values and can be considered as measures of the quantity of statistical information provided by those loci.

Another useful summary value is the largest eigenvalue of Σ_Θ. This can be considered a worst case measure because its statistical relevance is that it gives the maximum variance over all linear combinations $\Sigma_{j=1}^{S} a_j\hat{\theta}_j$, where $a = (a_1, \ldots, a_S)$ is such that $\Sigma_{j=1}^{S} a_j^2 = 1$ (Strang 1980). (Therefore it can be used to derive an upper bound on the variance of estimates of the combined contribution from two or more stocks, or of the difference in contribution between two stocks.) This eigenvalue is used in the Numerical Results section and is denoted by $\eta_X(\Theta)$ where the subscript X acts as an index representing the loci being assayed. It is calculated with mixed fishery sample size N set to 1, since it was shown in the Theory section that sample size simply acts on Σ_Θ as a constant of proportionality. Thus the performance measure is $1/\eta_X(\Theta)$. The exact relationship between this measure and the measure $\zeta_X(\Theta)$ used by GBM is given in the Appendix.

Numerical Results

Average Performance over All Possible Compositions

Table 1 gives the performance value $(\mathrm{Avg}_\Theta(\eta_X))^{-1}$ for 10 diallelic loci when used individually in solving a three-stock mixture. The 10 loci used correspond to those investigated by GBM in their table 3 and the average is evaluated over the same fine uniform grid of Θ vectors (increment length 0.005). (Table 3 of GBM presents results for 20 loci, but by relabelling of the stocks (1, 2, and 3) and alleles (a and A) it is clear that it is necessary to consider only these 10 distinct loci.)

The rankings provided by $(\mathrm{Avg}_\Theta(\eta_X))^{-1}$ and $\mathrm{Avg}_\Theta(\zeta_X)$ are the same here. It is seen below that this is not the case in general

TABLE 1. Average and minimum performance (over a uniform grid of all possible three stock compositions) of 10 diallelic loci applied to three stock mixtures. The quantity p_j is the probability of allele A in stock j. $\text{Avg}_\Theta(\zeta_X)$ is the utility measure used by GBM.

Locus	p_1	p_2	p_3	$(\text{Avg}_\Theta(\eta_X))^{-1}$	$\text{Min}_\Theta(\eta_X^{-1})$	$\text{Avg}_\Theta(\zeta_X)$
1	0	0.6	1	1.1437	0.5829	1.0818
2	0	0.2	1	0.6170	0.2498	0.7572
3	0	0.4	0.8	0.3415	0.2143	0.3885
4	0.2	0.8	1	0.2213	0.1232	0.2571
5	0	0.6	0.8	0.1533	0.1015	0.1904
6	0	0.2	0.6	0.0946	0.0424	0.1266
7	0	0.4	0.6	0.0712	0.0390	0.0923
8	0.2	0.6	0.8	0.0699	0.0641	0.0696
9	0.6	0.8	1	0.0309	0.0139	0.0433
10	0.2	0.4	0.6	0.0184	0.0139	0.0187

TABLE 2. The entry in row i and column j is the percentage of times that locus j does better than locus i (i.e. has smaller $\eta_X(\Theta)$ than locus i) over a uniform grid of all possible three stock compositions.

	1	2	3	4	5	6	7	8	9	10
1	—	0	0	0	0	0	0	0	0	0
2	100	—	14	0	0	0	0	0	0	0
3	100	86	—	28	0	9	4	0	1	0
4	100	100	72	—	29	0	0	0	0	0
5	100	100	100	71	—	34	16	0	4	0
6	100	100	91	100	66	—	0	25	0	0
7	100	100	96	100	84	100	—	49	0	0
8	100	100	100	100	100	75	51	—	12	0
9	100	100	99	100	96	100	100	88	—	0
10	100	100	100	100	100	100	100	100	100	—

and that $\zeta_X(\Theta)$ may not give the correct rankings when it is evaluated at fixed values of Θ and therefore also when evaluated on restricted ranges of Θ.

Figure 3 of GBM shows results of simulations used to compare the predicted rankings with the average composition estimator precision obtained when using each of those loci individually in a GSI study with mixed sample size 1000. An interesting feature of that figure is that loci 7 and 8 are seen to have nearly identical actual performance. One cannot infer this from the $\text{Avg}_\Theta(\zeta_X)$ values for these loci (0.0923 and 0.0696, respectively). However, it is predicted by the performance measure $(\text{Avg}_\Theta(\eta_X))^{-1}$ which gives very similar performance measures to loci 7 and 8 (0.0712 and 0.0699, respectively). Although locus 7 has slighter higher average performance it has considerably worse minimum performance (0.0390 versus 0.0641) than locus 8 and for this reason one might in practice favour locus 8 over locus 7.

The value $\text{Avg}_\Theta(\eta_X)$ is given by inverting the entries in the fifth column of Table 1. For loci 1 and 10 the values of $\text{Avg}_\Theta(\eta_X)$ are 0.874 and 54.318, respectively. That is, the largest eigenvalue of the covariance matrix expected from use of locus 1 will (on average over Θ) be approximately $0.874/54.318 \approx 0.016$ the magnitude of that expected from use of locus 10, assuming the same mixed fishery sample size. This implies that locus 1 can be expected to give estimates of composition that have $\sqrt{0.016} \approx 1/8$ the standard deviation of estimates obtained using locus 10.

Table 2 gives the percentage of compositions (on the uniform grid of possible Θ) for which each locus performs better than each other locus. For example, locus 7 performs better than locus 8 for 51% of the Θ vectors. Note that locus 1 dominates all other loci and that locus 10 is dominated by all other loci.

Performance at a Fixed Composition

In practice the researcher will often have some knowledge of the mixed fishery composition — possibly from GSI studies conducted in previous years. Since the performance of the assayed loci is a function of Θ, it makes sense to evaluate the performance measure at compositions that are believed to be close to the true composition.

We illustrate with the case where at least 90% of the mixed fishery is known to come from stock 3. Performance of the 10 loci used in Table 1 was evaluated for $\Theta = (0.05,0.05,0.9)$ and the more extreme value $\Theta = (0.01,0.01,0.98)$ (Table 3). The difference between GBM's $\zeta_X(\Theta)$ and the performance measure $1/\eta_X(\Theta)$ can be dramatic and $\zeta_X(\Theta)$ incorrectly ranks locus 2 above locus 1. As one might expect, it appears that the allele frequencies of the dominant stock largely dictate GSI performance. The three highest performance measures are for loci which are homozygous ($p_3 = 1$) in stock 3 and the three lowest measures for $p_3 = 0.6$.

The value $\Theta = (0.01,0.01,0.98)$ is very extreme, in the sense that it is close to the boundary of the feasible region, and for this reason the mixed fishery sample would probably have to be extremely large for the asymptotic covariance matrix Σ_Θ to be a good approximation to the true covariance matrix of Θ. Thus, for $\Theta = (0.01,0.01,0.98)$, the relative loci performance implied by Table 3 may not be realized except for very large sample sizes. To a lesser extent the same may be true for $\Theta = (0.05,0.05,0.9)$. To investigate this a simulation was conducted to see if the performance measure gives good measures of relative loci performance for moderate sample sizes. The simulation compares the relative performance of locus 1 and locus 4 when $\Theta = (0.05,0.05,0.9)$. The "control" experiment uses locus 1 and a mixed fishery sample size of 100 and we ask what sample size would be required to give the same estimate precision if locus 4 had been used instead of locus 1. By thinking of the performance measure $1/\eta_X(\Theta)$ as a measure of information content, we can say that the information value of locus 1 is 5.0819 and therefore a mixed fishery sample of size 100 has information content 508.19. Since the information value for locus 4 is 1.3698, it should require a mixed fishery sample of 371 fish to get the same information content ($371 \times 1.3698 = 508.20$).

The two experiments (locus 1 with a 100 fish mixed sample and locus 4 with a 371 fish mixed sample) are denoted by CONTROL and ASY, respectively. One hundred simulations were performed for each experiment and the resulting estimated compositions are displayed in Fig. 1. Experiments CONTROL

TABLE 3. Performance of 10 loci at compositions $\Theta = (0.05, 0.05, 0.90)$ and $\Theta = (0.01, 0.01, 0.98)$.

				$\Theta=(0.05,0.05,0.90)$		$\Theta=(0.01,0.01,0.98)$	
Locus	p_1	p_2	p_3	$\eta_X^{-1}(\Theta)$	$\zeta_{X'}(\Theta)$	$\eta_X^{-1}(\Theta)$	$\zeta_{X'}(\Theta)$
1	0	0.6	1	5.0819	1.0715	24.1130	1.0138
2	0	0.2	1	3.9711	1.1082	19.8491	1.0199
3	0	0.4	0.8	0.2246	0.2130	0.2161	0.2023
4	0.2	0.8	1	1.3698	0.8148	6.7867	0.9745
5	0	0.6	0.8	0.1095	0.1057	0.1059	0.1013
6	0	0.2	0.6	0.0458	0.0456	0.0427	0.0425
7	0	0.4	0.6	0.0415	0.0410	0.0392	0.0387
	0.2	0.6	0.8	0.0704	0.0691	0.0706	0.0689
9	0.6	0.8	1	0.2048	0.2000	1.0237	0.7479
10	0.2	0.4	0.6	0.0144	0.0143	0.0139	0.0139

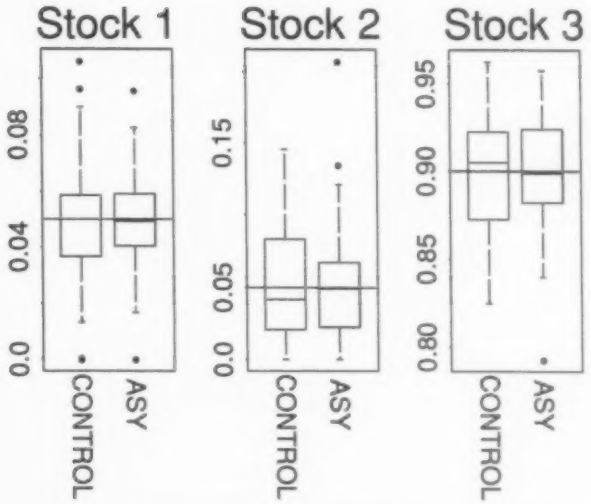

FIG. 1. Boxplots of the estimated compositions, from 100 simulations, of a three-stock mixture with true composition (0.05,0.05,0.90). The CONTROL study assays locus 1 and uses a mixed sample size of 100. The ASY study assays locus 4 and uses a mixed sample size of 371. The middle line in each box is the median value and the top and bottom of the box are the upper and lower quartiles, respectively. The height of the box is the interquartile range. The "whiskers" that extend from the top and bottom of each box reach to the furthest value that is within 1.5 times the interquartile range. Any values beyond that are displayed as dots.

and ASY produced estimates with similar precision — the total variances (sum of the diagonal elements of the observed covariance matrix) are 0.0028 and 0.0025 for these two experiments, respectively. This indicates that the sample sizes used were sufficiently large for the performance measures to be accurate.

A simpler but ad hoc procedure to indicate if sample size is sufficient is to calculate Σ_Θ and determine whether each θ_j is at least two standard deviations away from 0 and 1. If so, then the sample size is probably large enough.

Discussion

Whereas GBM speculated on the existence of some kind of functional relationship between their utility measure and composition estimator precision, this study proceeds directly and determines the covariance matrix, Σ_Θ, thereby allowing measures of loci performance to be directly related to estimator precision. Optimal design of GSI studies is immediate, since for different loci the ratio of their $1/\eta_X(\Theta)$ values gives their relative worth.

The methodology presented here assumes that the mixed sample size is sufficiently large for the asymptotic covariance matrix Σ_Θ to be a good estimator of the true covariance matrix of Θ. If sample size is small and Θ is close to the boundary of the feasible region (i.e. some θ_j are near-zero), then Σ_Θ is prone to overestimate the true variance of the composition estimator (Millar 1987). Millar (1987) showed that the infinitesimal jackknife estimator (Efron 1982) of the covariance matrix is easily calculated and provides superior estimates of composition estimator variance, especially when Θ is close to the boundary of the feasible region. However, although this estimator is analytic, it cannot be calculated until the mixed sample has been collected. Potential bias of the composition estimators is another reason to consider sample size. For moderate sample size it should not be a major concern, since the bias of maximum likelihood estimators decreases with increasing sample size more rapidly than does the standard deviation (Lehmann 1983).

Evaluation of Σ_Θ requires a summation over all possible genotypes (see equation (A.3)) and in the multilocus case this may be a daunting task, e.g. if 3 distinct alleles are possible at each locus (so that 6 distinct allele pairs are possible) and 10 loci are assayed, then the number of possible multilocus genotypes is $6^{10} = 60\ 466\ 176$. (In GSI analyses the asymptotic covariance matrix is typically evaluated using the observed mixed sample data instead of taking the expectation; see equation (A.5) in the Appendix and step 2 and remark 2 in the Theory section.) Moreover, the researcher may be choosing the best 10 loci to assay from a baseline collection of 20 loci, say, in which case there are 184 756 different 10-loci subsets to consider.

Although there does not appear to be any tractable way to infer the simultaneous performance of multilocus data from the performance of the loci individually, the magnitude of the calculation involved in the multilocus case may force the researcher to make an educated selection from the baseline collection on the basis of individual loci performance, or performance of all pairs of loci. In doing so it may be worth examining the entire covariance matrix Σ_Θ rather than just evaluating a summary value such as $\eta_X(\Theta)$. One might then be able to choose the subset of loci to be assayed so that all stock compositions can be estimated reasonably well. For example, this could prevent selection of a subset of loci where none of those loci ena-

bled good estimation of the contribution from one particular stock.

Acknowledgements

I am grateful to Marc Mangel, Richard Gomulkiewicz, and Jon Brodziak for extensive commentary on an earlier draft of this paper.

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Appendix

We use the notation of Millar (1987). In particular, S = number of stocks in the mixed fishery, N = size of mixed fishery sample, G = number of possible genotypes, x_{ij} = probability of genotype i within stock j, y_i = frequency of genotype i in the mixed fishery sample, and θ_j = proportion of stock j fish in the mixed fishery.

The log-likelihood function for the mixed fishery sample, written as a function of $\Theta = (\theta_1,\theta_2,\ldots,\theta_S)$ is

$$\text{(A.1)}\quad l(\Theta) = \sum_{i=1}^{G} y_i \log_e\left(\sum_{j=1}^{S} x_{ij}\theta_j\right).$$

The Theory section explained that precision of maximum likelihood estimators must be calculated using free parameters. Substituting θ_S with $1-\theta_1-,\ldots,-\theta_{S-1}$ in (A.1) gives the log-likelihood of the mixed fishery sample as a function of the free parameters $\Theta^* = (\theta_1,\theta_2,\ldots,\theta_{S-1})$:

$$\text{(A.2)}\quad l(\Theta^*) = \sum_{i=1}^{G} y_i \log_e\left(x_{iS} + \sum_{j=1}^{S-1}(x_{ij}-x_{iS})\theta_j\right).$$

The information matrix $I(\Theta^*)$ obtained from $l(\Theta^*)$ has (k,l)th element

$$\text{(A.3)}\quad I(\Theta^*)_{kl} = \sum_{i=1}^{G}\left(\frac{(x_{ik}-x_{iS})(x_{il}-x_{iS})}{x_{iS} + \sum_{j=1}^{S-1}(x_{ij}-x_{iS})\theta_j}\right)$$

for $k = 1,2,\ldots,S-1$, $l = 1,2,\ldots,S-1$.

Define Q to be an $S \times (S-1)$ matrix with upper $(S-1) \times (S-1)$ submatrix being the identity matrix and with the elements of the Sth row all being -1. For example, for a three-stock mixture:

$$Q = \begin{pmatrix} 1 & 0 \\ 0 & 1 \\ -1 & -1 \end{pmatrix}.$$

The asymptotic covariance matrix of Θ^* is $\Sigma_{\Theta^*} = I^{-1}(\Theta^*)/N$, and from Seber 1977 (Theorem 1.4, p. 10) the asymptotic covariance matrix of Θ is given by

$$\text{(A.4)}\quad \Sigma_\Theta = Q\Sigma_{\Theta^*}Q^T = QI^{-1}(\Theta^*)Q^T/N.$$

Remark: In practical GSI application the information matrix is calculated using the mixed sample data because summation over all possible multilocus genotypes may be computationally impossible. This gives the observed information matrix, with (k,l)th element

$$\text{(A.5)}\quad I^{\text{obs}}(\Theta^*)_{kl} = \frac{1}{N}\sum_{r=1}^{N}\left(\frac{(x_{i_r k}-x_{i_r S})(x_{i_r l}-x_{i_r S})}{(x_{i_r S} + \sum_{j=1}^{S-1}(x_{i_r j}-x_{i_r S})\theta_j)^2}\right)$$

where i_r is the genotype of the rth fish in the mixed sample.

In essence, GBM evaluated the overparametrized $S \times S$ information matrix with (k,l)th element

$$I(\Theta)_{kl} = \sum_{i=1}^{G}\left(\frac{x_{ik}x_{il}}{\sum_{j=1}^{S} x_{ij}\theta_j}\right) \quad k=1,\ldots,S,\ l=1,\ldots,S$$

and used $\zeta_X(\Theta)$ (the smallest eigenvalue of $I(\Theta)$) as their measure of baseline data utility. It can be verified directly, or obtained from Lehmann (1983, p. 127), that $I(\Theta^*) = Q^T I(\Theta)Q$. Thus, from (A.4), $\Sigma_\Theta = Q(Q^T I(\Theta)Q)^{-1}Q^T/N$.

GBM's argument for using the smallest eigenvalue of $I(\Theta)$ is that it gives the smallest curvature of the log-likelihood function (A.1). In geometric terminology, this is written (Strang 1980, p. 266)

$$\min_{\|\alpha\|\le\delta}\frac{\alpha^T I(\Theta)\alpha}{\alpha^T\alpha} = \zeta_X(\Theta)$$

where the minimization is over all nonzero vectors $\alpha = (\alpha_1,\ldots,\alpha_S)$ such that $\|\alpha\| = (\sum_{j=1}^{S}\alpha_j^2)^{1/2} < \delta$ for any arbitrarily small positive δ. Since $I(\Theta)$ is a positive definite matrix, $\zeta_X(\Theta)$ is positive (Strang 1980, p. 250). In the GSI application, $\alpha = (\Theta - \hat{\Theta})$ (see equation (3) of GBM) and so the elements of α sum to zero, but this constraint was not taken into consideration.

To add the constraint into the curvature argument, define $P = I_S - J_S/S$ where I_S is the $S \times S$ identity matrix and J_S is the $S \times S$ matrix having 1 for all its elements. For example, when $S = 3$:

$$P = \frac{1}{3}\begin{pmatrix} 2 & -1 & -1 \\ -1 & 2 & -1 \\ -1 & -1 & 2 \end{pmatrix}.$$

If α is any S-dimensional vector, then $P\alpha$ has sum zero. Moreover, if α has sum zero, then $P\alpha = \alpha$. (In linear algebra, P is called a projection matrix). Thus, the minimum curvature of the log-likelihood function, subject to α summing to zero, is given by

$$\min_{\|\alpha\|<\delta} \frac{\alpha^T PI(\Theta)P\alpha}{\alpha^T\alpha}$$

which is given by the smallest eigenvalue of $PI(\Theta)P$ and will be denoted by $\zeta^*_\chi(\Theta)$. This value gives the minimum curvature of the log-likelihood surfaces displayed in fig. 1 and 2 of GBM.

It is a matter of linear algebra to show that $PI(\Theta)P$ is the Moore–Penrose generalized inverse (Seber 1977) of $Q(Q^T I(\Theta)Q)^{-1}Q^T = \Sigma_\Theta$ (calculated with N set to 1). An immediate consequence is that $\zeta^*_\chi(\Theta) = \eta_\chi^{-1}(\Theta)$. That is, the utility measure that has been used throughout this paper ($\eta_\chi^{-1}(\Theta)$) and which was derived from the asymptotic covariance matrix is also the measure that is obtained from correct application of the curvature argument.

Feeding Habits of Northeast Atlantic Harp Seals (*Phoca groenlandica*) along the Summer Ice Edge of the Barents Sea

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Lydersen, C., L. A. Angantyr, Ø. Wiig, and T. Øritsland. 1991. Feeding habits of Northeast Atlantic harp seals (*Phoca groenlandica*) along the summer ice edge of the Barents Sea. Can. J. Fish. Aquat. Sci. 48: 2180–2183.

Stomachs from 58 harp seals (*Phoca groenlandica*) from the northern part of the Barents Sea were collected between August 20 and September 5, 1987. Fifty-six of the stomachs contained identifiable remains. The amphipod *Parathemisto libellula* was the most common food item, found in 98% of the seal stomachs and constituting 57.9% of the total volume. Fish were the second most important prey group, with Arctic cod (*Boreogadus saida*) as the dominant species followed by Nybelin's sculpin (*Triglops nybelini*) and Greenland halibut (*Reinhardtius hippoglossoides*). Decapods, mainly *Pandalus borealis*, were also common as prey of harp seals. No sex- or age-related differences in choice of food were found. From knowledge of the depths at locations where seals were collected and the presence of fresh benthic fishes in the seal stomachs, it is assumed that harp seals could forage at depths below 300 m.

On a recueilli 58 estomacs de phoques du Groenland (*Phoca groenlandica*) capturés dans le secteur nord de la mer de Barents entre le 20 août et le 5 septembre 1987. De ceux-ci, 56 contenaient des restes identifiables. L'amphipode *Parathemisto libellula* était l'aliment le plus fréquemment consommé, étant retrouvé dans 98 % des estomacs et constituant 57,9 % du volume total. Le poisson venait au deuxième rang des proies les plus recherchées; le saïda franc *Boreogadus saida* était l'espèce la plus commune, puis venaient le faux-trigle aux grands yeux *Triglops nybelini* et le flétan du Groenland *Reinhardtius hippoglossoides*. Des décapodes, en particulier *Pandalus borealis*, étaient aussi une proie commune. On n'a pas relevé de différences liées au sexe et à l'âge en ce qui a trait au choix d'aliments. En se basant sur les profondeurs aux sites de capture des phoques et la présence de poissons benthiques frais dans les estomacs étudiés, on formule la conclusion que le phoque du Groenland peut plonger jusqu'à plus de 300 m à la recherche de nourriture.

Received June 25, 1990
Accepted May 1, 1991
(JA626)

Reçu le 25 juin 1990
Accepté le 1 mai 1991

The harp seal (*Phoca groenlandica*) is an important species in the marine ecosystem in the Barents Sea. Its impact on the system is dependent on the size of the population and its food habits. No recent estimate of the population size exists. In 1978, the population was estimated as 800 000 seals with an annual increase of about 5% (Benjaminsen 1979). During the early 1980's, the population size was believed to exceed 1 million, but more recent studies indicate that it is probably declining (Ulltang and Øien 1988).

Markussen and Øritsland (1985) estimated that a population of 1 million harp seals in the Barents Sea needs 1.4–4.2 million tons of food every year depending on the type of prey consumed. However, knowledge of the feeding habits of Northeast Atlantic harp seals is scarce. Much of the available information is based on material collected during the breeding season or deals with the Northwest Atlantic stock (Sivertsen 1941; Myers 1959; Sergeant 1973; Bowen 1985; Kapel and Angantyr 1989; Finley et al. 1990). This paper reports on feeding habits of harp seals collected along the summer ice edge of the Barents Sea.

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Material and Methods

Stomachs of 58 harp seals were collected at the ice edge in the northern parts of the Barents Sea between Svalbard and the Franz Josef Land archipelagoes (79°15′–79°55′N, 27°35′–44°50′E) from August 20 to September 5, 1987. The seals were shot in the water and dissected immediately. The stomach contents were rinsed on a sieve with a mesh size of 1 mm and then frozen. Teeth and sex organs were sampled to enable grouping of the seals into adults and subadults. Cross-sections 0.2 mm thick were cut from the canines using a modification of the double-bladed cutting machine described by Khutzin (1967). Sections were mounted on glass microscope slides, and dentin growth layers were counted in transmitting light using a binocular dissecting microscope. All sections were read twice by two different readers, and no difference between readings concerning which of the adult or subadult group they belonged in was found. Presence of corpora lutea or albicantia in ovaries was the criterion for sexual maturity in females. Based on comparisons of relative size of testis, body size of the animal, and results from the age determination, males 6 yr or older were considered sexually mature and 5 yr and younger as subadults. Water depth at most of the hunting sites was recorded using an echo-sounder.

Stomach contents were sorted to the lowest possible taxonomic level. Total wet mass of the contents and volume

TABLE 1. Distribution of major groups of preys from stomachs of harp seals collected in northern Barents Sea during the autumn of 1987.

Prey group	Volume percent (*V*)	Occurrence (*O*)	Abundance factor (*A*)
Fish	28.0	0.70	19.6
Amphipods	57.9	0.98	56.7
Decapods	13.1	0.66	8.6
Cephalopods	1.0	0.29	0.29

percentages (*V*) of the major prey groups were recorded. A nontrace frequency of occurrence (*O*) was calculated for the main prey groups (Bigg and Perez 1985). Prey represented by trace occurrences only, such as beaks of squid and otoliths of fish, were excluded from these calculations and were only examined for qualitative purposes. To evaluate the relative importance of different prey, an abundance factor (*A*) was calculated as $A = V \times O$.

Results

Two stomachs were empty, and the mean wet mass of the remaining 56 stomach contents was 273 ± 253 g (SD) with a range of 0.1–1326 g.

Amphipods were the dominant prey group (Table 1) and consisted almost entirely of *Parathemisto libellula* (Table 2). Fishes constituted the second most important group. The largest fraction of the fish group, however, consisted of partly digested specimens which were not identified to species level. The identifiable fraction was dominated by Arctic cod (*Boreogadus saida*). Benthic fish like Greenland halibut (*Reinhardtius hippoglossoides*) and Nybelin's sculpin (*Triglops nybelini*) were also common. Some invertebrate species were found only as traces in single seal stomachs and were excluded in Table 2. They include the copepod *Pareuchaeta glacialis*, the isopod *Idotea granulosa*, and the bivalves *Astarte elliptica* and *Leda pernula*.

The harp seal sample consisted of 23 females and 35 males. The mean age of females was 7.2 ± 4.7 yr (SD) (range 1–16 yr) and of males was 8.1 ± 4.5 yr (range 1–15 yr). To test for age- and/or sex-related differences in diet, we grouped the stomach contents of adult males ($N = 23$), subadult males ($N = 12$), adult females ($N = 11$), and subadult females ($N = 12$), respectively (Fig. 1). No significant difference in diet was found between males and females ($\chi^2 = 0.190$, $p = 0.66$) or between adults and subadults ($\chi^2 = 0.365$, $p = 0.55$). The greatest difference was found between subadult females and adult females (Fig. 1), but was not significant ($\chi^2 = 2.318$, $p = 0.13$).

The mean depth recorded at locations where stomachs contained freshly eaten benthic fishes ($N = 12$) was 256 ± 61 m, with a maximum recorded depth of 340 m.

Discussion

The main potential bias in using volumetric assessment of feeding habits is the effect of progressive digestion. Food items which are digested more quickly or are eaten earlier than others will be underrepresented. Fish species found in stomachs containing a greater volume of prey are likely to be overrepresented, since digestion is less advanced and specimens more identifiable than in samples from small stomachs. Since our sample consists of many animals with small amounts of food in their stomachs, this may lead to a biased assessment of the fish composition of the diet. The main bias using the nontrace frequency of occurrence is that small specimens are given an exaggerated importance. When multiplying the volume per-

TABLE 2. Occurrence of prey species within each main prey group from stomachs of harp seals collected in the northern Barents Sea during the autumn of 1987.

Prey species	Volume percent (*V*)	Occurrence (*O*)	Abundance factor (*A*)
Fish			
Unidentified fish remains	63.7		
Boreogadus saida	20.4	0.36	7.3
Triglops nybelini	8.4	0.27	2.3
Reinhardtius hippoglossoides	6.1	0.18	1.1
Other fish including *Sebastes marinus*, *Hippoglossoides platessoides*, *Leptoclinus maculatus*, *Benthosema glaciale*, and *Liparis* sp.	1.4	0.12	0.2
Amphipods			
Parathemisto libellula	99.6	0.98	97.6
Gammarus spp.	0.2	0.07	0.01
Other amphipods	0.2	0.02	0.003
Decapods			
Pandalus borealis	95.0	0.66	62.7
Sabinea septemcarcinatus	2.0	0.13	0.26
Other decapods including *Lebbeus polaris* and *Pasiphaea tarda*	3.0	0.09	0.27
Cephalopods			
Gonatus fabricii	83.3	0.27	22.5
Octopods including *Bathypolipus arcticus* and *Elodone cirrhosa*	16.7	0.04	0.7

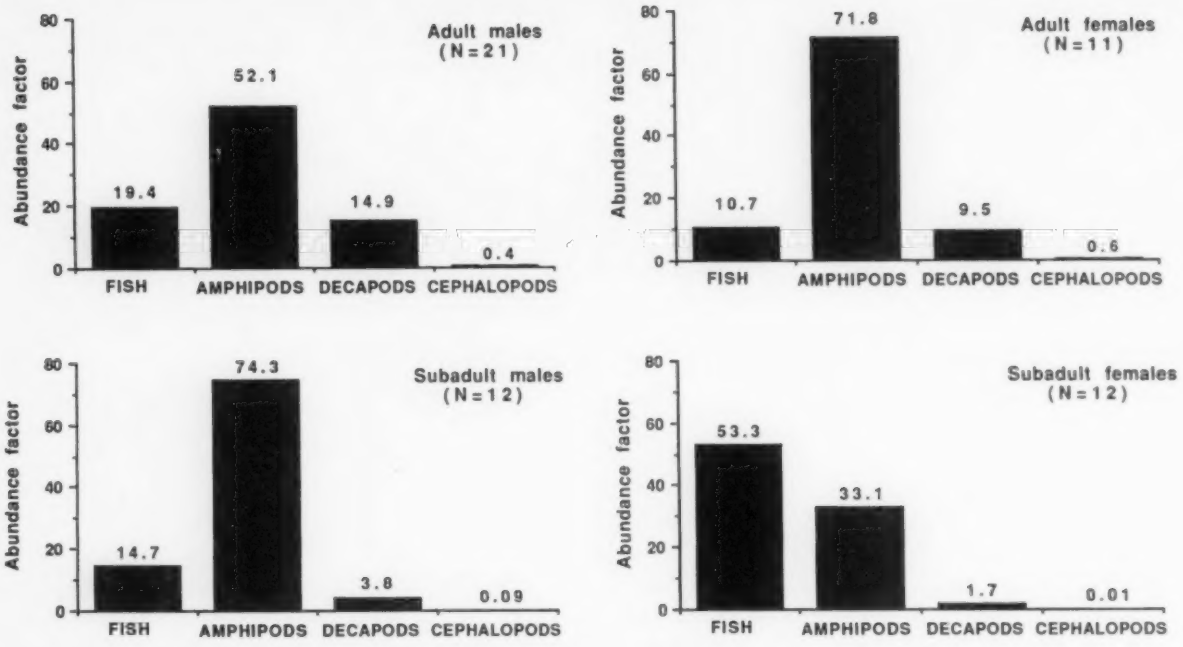

FIG. 1. Results from analysis of stomach contents of harp seals of different age and sex groups collected in the Barents Sea, August 1987.

centage with the frequency of occurrence, we moderate the effect one large prey organism in one single stomach would have on the total assessment.

Most information on harp seal diets is collected from their wintering grounds. On diet of the Northeast Atlantic population, Smirnov (1924) stated, without providing details, that pelagic crustaceans and Arctic cod followed by capelin (*Mallotus villosus*), herring (*Clupea harengus*), and pelagic molluscs were the most important food items. Sivertsen (1941) found that, after weaning, harp seal pups feed on pelagic crustaceans, especially the euphausids *Thysanoessa inermis* and *Thysanoessa raschii* and the amphipod *Anonyx nugax*. Yearlings had a different diet including capelin and shrimp species such as *Spirontocaris turgida* and *Crangon crangon*. Information on adult diet was based on two seals with contents in their stomachs which contained *T. raschii* and *C. crangon*.

For the Northwest Atlantic population, Myers (1959) found fish remains, with herring as the dominant species, in a sample of 185 harp seals from Canadian waters. Sergeant (1973) stated that pelagic fishes, especially capelin, plus pelagic and benthic crustaceans were the most important prey. He collected a small sample from harp seals summering in the cold waters between northwest Greenland and the Canadian Arctic archipelago, and these seals were mainly eating Arctic cod and various crustaceans including *P. libellula*. Bowen (1985) reviewed available data on feeding of the Northwest Atlantic harp seal population and concluded that they fed mainly on pelagic fishes dominated by capelin and Arctic cod and on a variety of invertebrates, particularly euphausids and shrimps. Kapel and Angantyr (1989) studied stomachs from 661 harp seals collected at different locations along the west coast of Greenland during 1985–88. They found capelin to be the dominant prey in spring and summer in southwest and central-west Greenland. In the north and northwest of Greenland, however, Arctic cod and polar cod (*Arctogadus glacialis*) and crustaceans, including *P. libellula*, dominated. Finley et al. (1990) found that the main prey of summering harp seals in the Canadian high Arctic was Arctic cod, with polar cod as the second most important prey group. Invertebrates were of minor importance in their sample of 157 seal stomachs but were dominated by *Parathemisto* spp. and *Mysis* spp.

The results of the present study are in general agreement with studies of harp seals feeding in other Arctic areas (Sergeant 1973; Kapel and Angantyr 1989; Finley et al. 1990). Comparing results from investigations of stomach contents from different marine mammals which, at least partly, forage pelagically in association with the ice edge both in the European and the American Arctic, it appears that pelagic crustaceans including *P. libellula* and Arctic cod are the most significant prey (Lowry et al. 1980; Bradstreet and Cross 1982; Finley and Gibb 1982; Bradstreet et al. 1986; Gjertz and Lydersen 1986; Smith 1987; Kapel and Angantyr 1989; Lydersen et al. 1989). The sample of Finley et al. (1990) was totally dominated by Arctic cod. Their sample was collected in coastal waters, while our sample was collected in a more pelagic situation. Since *P. libellula* is a pelagic amphipod, this may explain this difference in the diet.

During summer, large herds of harp seals are normally observed in open waters between the coast of Finmark (northern Norway) and the Svalbard – Franz Josef Land archipelagoes. Considerable effort was expended to locate some of these herds, including assistance from the large fleet of fishing vessels in the area. However, no herds of harp seals were found. In general, the only area where seals were observed was along the ice edge, and sampling was therefore concentrated in this area. One explanation for the scarcity of seals in open water could be the collapse in the stock of capelin in the Barents Sea in 1987 (Anonymous 1990). It is assumed that capelin has been the

principal food source for harp seals summering in this area (Wiig 1988) and that the collapse induced the seals to search for food in other areas. One harp seal was shot in the southern Barents Sea but was excluded from our analysis, since it had been collected remotely from the rest of the sample. This seal, however, had eaten capelin.

Sergeant (1973) suggested diving abilities of harp seals down to 250 m based on the occurrence of fresh benthic fishes in the stomachs of seals collected in areas of known depths. We collected harp seals containing fresh benthic fishes in areas where depth exceeded 300 m. *Reinhardtius hippoglossoides* was one of the species considered benthic. This species has, however, been found to have a bathypelagic mode of life (Chumakov 1969), feeding in the water column (Haug and Gulliksen 1982), and might therefore seem unreliable as an indicator of foraging depths of harp seals. Most of the *R. hippoglossoides* in the Svalbard area are, however, caught at depths below 200 m (Godø and Haug 1987), and Chumakov (1969) stated that *R. hippoglossoides* are on the bottom during daytime, when most seals were collected for this study. The results therefore indicate that the harp seal may feed at depths of 300 m.

In conclusion, amphipods, mainly *P. libellula*, were found to be the most significant food item of harp seals feeding along the ice edge in the northern parts of the Barents Sea. Fish were found to be the second most important prey, with Arctic cod as the dominant species followed by *T. nybelini* and *R. hippoglossoides*. No significant sex- or age-related difference in choice of prey was found.

Acknowledgements

This project was financed by "Fondet for Fiskeleting og Forsøk" through the Institute of Marine Research, Directorate of Fisheries in Bergen, Norway. We would like to thank Yu. I. Nazarenko and the crew onboard "Veslekari" for their help during the field work.

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Mathematical Analysis of Temporal and Spatial Trends in the Benthic Macroinvertebrate Communities of a Small Stream

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Matthews, G. B., R. A. Matthews, and B. Hachmöller. 1991. Mathematical analysis of temporal and spatial trends in the benthic macroinvertebrate communities of a small stream. Can. J. Fish. Aquat. Sci. 48: 2184–2190.

Macroinvertebrates were collected at four sites in Padden Creek, a small second-order stream in Whatcom County, Washington, USA. Two upstream sites were characterized by high densities of sensitive taxa, predominantly mayflies, stoneflies, and caddisflies, and two downstream sites showed high densities of tolerant taxa, especially true flies, annelids, *Baetis* mayflies, and gastropods. Despite the small sample size, some statistical techniques proved useful. The first two components of correspondence analysis were used to confirm the existence of both seasonal and spatial trends in the benthic macroinvertebrate populations of the stream. Neither component alone, however, ordinated the samples with respect to these trends. Combinations of the first two components were required. A standard clustering technique, *k*-means clustering with squared Euclidean distance, further confirmed the seasonal trend. Nonmetric clustering, not widely used in the analysis of ecological data, was necessary to confirm the spatial trend. Nonmetric clustering was also able to identify a small number of "significant" taxa, i.e. taxa that reliably served as indicators of spatial position on the stream.

On a effectué un échantillonnage des macroinvertébrés à quatre sites du ruisseau Padden, un petit cours d'eau de second ordre situé dans le comté Whatcom de l'État de Washington (É-U). Des densités élevées de taxons sensibles étaient caractéristiques des deux sites d'amont, en particulier des éphémères, des perles et des phryganes, tandis que les deux sites d'aval abritaient des densités élevées de taxons tolérants, surtout des mouches, des annélides, des éphémères du genre *Baetis* et des gastéropodes. Malgré la faible taille des échantillons, certaines méthodes statistiques se sont révélées utiles. Ainsi, les deux premières composantes de l'analyse factorielle de correspondance ont permis de confirmer l'existence de tendances saisonnières et spatiales dans les populations de macroinvertébrés benthiques du cours d'eau. Toutefois, ni l'une ni l'autre de ces composantes n'a permis d'effectuer une ordination des échantillons en ce qui concerne ces tendances, ordination obtenue toutefois par la combinaison des deux premières composantes. L'agglomération de moyennes *k* couplée à la distance euclidienne au carré, une technique agglomérative normalisée, a permis d'étayer cette tendance saisonnière. L'agglomération non métrique, rarement utilisée dans l'analyse de données écologiques, a été nécessaire pour confirmer la tendance spatiale. Cette dernière analyse a aussi permis d'identifier un faible nombre de taxons "significatifs", c'est-à-dire des taxons qui ont servi d'indicateurs fiables de la position spatiale dans le cours d'eau.

Received September 9, 1990
Accepted May 2, 1991
(JA718)

Reçu le 9 septembre 1990
Accepté le 2 mai 1991

One of the fundamental principles of mathematical ecology is that changes in the statistical makeup of the biota are reflections of changes in the physical environment. The dominance of certain taxa at a particular site or between sites can serve as a quantifiable record of the strength and direction of environmental changes (Faith and Norris 1989). In the ecology of streams, there are often two dominant environmental changes, one associated with time and the other with location (Green 1974). The benthic community varies with the season, and also with its spatial position in the stream. Many benthic macroinvertebrates have habitat requirements that correspond to longitudinal gradients, upstream to downstream. For example, because they require highly oxygenated waters, many stoneflies are restricted to headwater streams, which are often less polluted and more turbulent than downstream reaches (Hynes 1970; McCafferty 1981). Many other stream characteristics can be viewed as changing along this longitudinal gradient, due to the unidirectional downstream flow. This view of streams as gradients has influenced many of the fundamental theories on how streams function, including organic matter processing, macroinvertebrate community trophic structure, instream primary productivity, and nutrient cycling (see Minshall 1988 and Fisher 1983 for general reviews). However, the complex distributions and patterns exhibited by macroinvertebrates

make statistical confirmation of such relationships difficult. The problem of identifying reliable taxonomic indicators of environmental changes is even more difficult.

In this paper we used ordination by correspondence analysis and clustering by two techniques, *k*-means clustering and nonmetric clustering, to obtain statistical confirmation of the benthic macroinvertebrate response to both the longitudinal and the seasonal trends. Correspondence analysis is well documented in the literature (e.g. Gauch et al. 1977; Kenkel and Orloci 1986; ter Braak 1986), but, while ordination has been used extensively for finding and confirming terrestrial vegetation gradients (e.g. Minchin 1987), it has been used much less frequently to examine gradients in stream data (e.g. Green 1974; Culp and Davies 1980; Sheldon and Haick 1981; Schaeffer and Perry 1986; Faith and Norris 1989). *K*-means clustering is also widely used in many fields (Jain and Dubes 1988). Nonmetric clustering, described in the Appendix, is a new technique and has not been widely applied to ecological data although we have found it useful in a variety of aplications (Matthews and Hearne 1991; Mathews et al. 1991). We found that the combination of these three analytical techniques provided an excellent approach to our data set. The spatial and temporal trends were both revealed by correspondence analysis. The temporal trend was confirmed by *k*-means clustering which successfully separated samples by date, and the spatial trend was similarly confirmed by the nonmetric clustering which successfully separated samples by site.

The data we used for our analyses were collected from Padden Creek, a small second-order stream located adjacent to the city of Bellingham in Whatcom County, Washington. Hachmöller (1989) and Hachmöller et al. (1990) found that the macroinvertebrate fauna in Padden Creek showed distinct upstream and downstream distribution patterns. These distribution patterns were thought to be related to differences in the riparian community, especially canopy cover, and the input of nonpoint-source runoff from residential and agricultural areas, which created a turbid, nutrient-enriched "lower reach" in the creek.

Methods

Macronvertebrate Sampling

Four sites were sampled in Padden Creek (Fig. 1). Site 1 was located approximately 1 km downstream from the Lake Padden outfall in a forested, relatively undisturbed area. Site 2 was located in a channelized reach that had a less diverse substrate than Site 1. Both Sites 1 and 2 were upstream from the confluence of Padden and Connelly Creeks. Connelly Creek is a nutrient-enriched tributary that drains agricultural and residential lands. Site 3 was located about 1.5 km downstream from Connelly Creek in a forested city park that was more disturbed than Site 1. Site 4 was located in a freshwater wetland close to the mouth of Padden Creek. Based on vegetation, water quality, and substrate sampling, Hachmöller et al. (1990) and Uhlig (1991) characterized the four sites as in Table 1.

The macroinvertebrate samples were collected monthly at each site from June through October 1988 using a Surber sampler (1-mm net mesh). Ten samples were collected at each site on each date. The invertebrates were keyed to the lowest practical taxon (genus in most cases) using the following references: Anderson (1976), Edmunds and Jensen (1976), Hatch (1953–65), Jewett (1959), Merritt and Cummins (1984), Pennak (1978), Ricker and Scudder (1975), Ross (1937), Stark and Gaufin (1976), and Stone et al. (1965). Macroinvertebrate densities for each taxon were calculated as the average number of individuals per square metre ($n = 10$ per site and date).

Statistical Tests

Throughout this section, a "sample" refers to the pooled macroinvertebrate densities at a unique site and date; there were 20 samples in this study (4 sites × 5 dates). Individual macroinvertebrate densities for each taxon are called "replicates." There were 10 replicates for each taxon (63 taxa) at each date and time (a maximum of 12 600 replicates, many of which had values of zero). Some statistical tests were performed on both the sample data averaged by replicate and the raw data, not averaged by replicate; however, only the results from the averaged sample tests are reported here. Generally, as might be expected, the raw data yielded similar results, but with larger variances.

We ordinated the samples using correspondence analysis. Correspondence analysis (also called reciprocal averaging) determines taxa scores and sample scores in an "uninformed" manner, i.e. without prior grouping of the samples. Thus, samples are ordinated independently of information regarding the actual site or date at which they were collected. For our purposes, it was important that the correspondence analysis procedure give several ordinations of the samples (first, second, third components, etc.), for we found that two components were necessary to reveal trends indicated by our subjective evaluations. The correspondence analysis procedure is similar to principal components and factor analysis, but has been shown to be superior to these methods in typical environmental data sets (Kenkel and Orloci 1986; Ludwig and Reynolds 1988).

The data were also clustered by the *k*-means algorithm using squared Euclidean distance, and nonmetric clustering. *K*-means clustering (Jain and Dubes 1988) views the samples as points in *n*-dimensional space, where *n* is the number of taxa. It seeks "clusters" of samples such that the distance between samples from the same cluster is generally less than the distance between samples from different clusters. The measure of distance between samples is called the metric. A clustering is optimal in the metric sense if it maximizes the difference between the average intracluster distance and the average intercluster distance. There are many measures of "distance" for samples, and the choice of a particular distance metric can have a radical effect on the resulting clusters. For our *k*-means clustering we used squared Euclidean distance. Nonmetric clustering, described in the Appendix, is a new procedure that does not use a distance metric to determine clusters (Matthews and Hearne 1991). Instead, a clustering is optimal in the nonmetric sense if it maximizes the association between clusters and a large number of taxa. Each taxon is also given a "score" by nonmetric clustering, which is a measure of how strongly that particular taxon is associated with the clustering. Both nonmetric and *k*-means clustering are uninformed procedures, like correspondence analysis, and do not require prior grouping of samples.

Correspondence analysis, metric clustering, and nonmetric clustering were also used in an effort to identify diagnostic taxa, i.e. a subset of the taxa that could be used as indicators of environmental conditions. Correspondence analysis not only ordinates the samples, but also ordinates the taxa, and thus "large" taxa scores might be taken to indicate taxa important

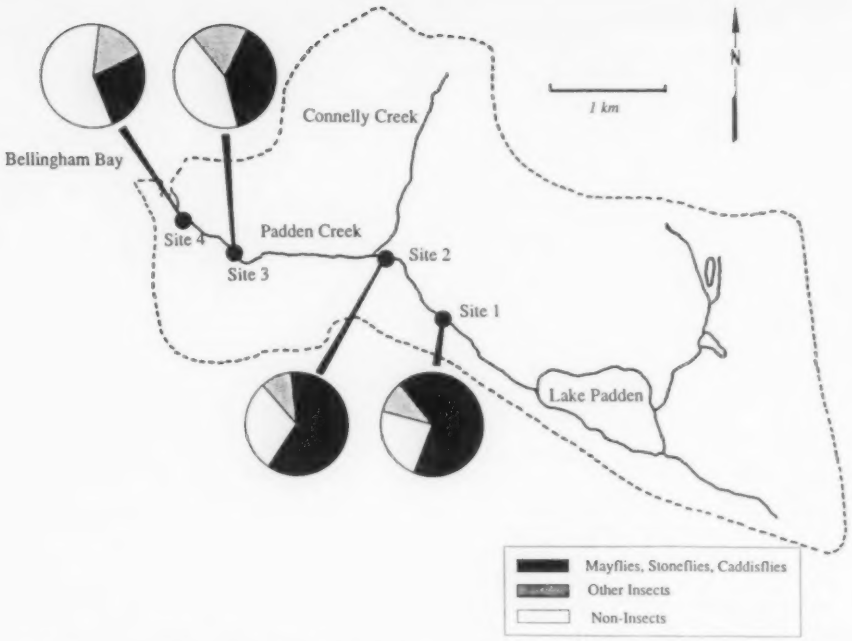

FIG. 1. Padden Creek sampling sites and relative proportions of major macroinvertebrate taxa. The macroinvertebrate proportions were calculated by averaging the macroinvertebrate densities (no./m^2) at each site for the entire study period.

TABLE 1. Characterization of the four sampling sites.

Factor	Site 1	Site 2	Site 3	Site 4
Nutrient concentration	Low–moderate	Low–moderate	Elevated	Elevated
Riparian vegetation	Second-growth coniferous forest	Alder; gaps in canopy	Second-growth coniferous forest	Freshwater wetland
Stream gradient	61 m/km	19 m/km	8 m/km	11 m/km
Substrate	Diverse cobble–pebble	Uniform cobble–pebble	Diverse pebble–sand	Diverse pebble–sand

to the correspondence analysis ordination. K-means clustering does not rank taxa in importance, and so was not used to identify diagnostic taxa. Nonmetric clustering, however, is designed to cluster data and simultaneously identify the taxa that are "important" with respect to these clusters (Matthews and Hearne 1991). In this regard it is similar to conceptual clustering techniques (Fisher and Langley 1986), which not only cluster the data, but attempt to show how those clusters can be characterized by a small subset of the data parameters. A nonmetric clustering which is meaningfully related to a spatial or longitudinal trend will also give a list of important taxa, which could be used as indicators of that trend.

Results

Hachmöller (1989) and Hachmöller et al. (1990) found that the most abrupt change in macroinvertebrate community structure occurred between Sites 2 and 3, which was attributed primarily to the influence of Connelly Creek. These changes can be seen in the pie charts summarizing the benthic community in Fig. 1. Mayflies, stoneflies, and caddisflies were collected in greater densities at the upstream sites (Sites 1 and 2); these three orders made up 62–67% of the macroinvertebrate densities at the upstream sites, but only 26–40% of the densities at the downstream sites (Sites 3 and 4). In addition, many of the uncommon taxa (less than 0.5% of the total density) were collected more frequently at the upstream sites, especially large, predatory stoneflies. This may be an artifact of the taxonomic technique because not all taxa were identified to the same level. In particular, Chironomidae and many of the noninsect taxa were identified only to family. This is a pervasive taxonomic dilemma, and its relevance to our statistical tests will be discussed below. In general, the macroinvertebrates collected at the downstream sites were mostly taxa having relatively cosmopolitan distributions such as *Baetis* and Chironomidae and included a large proportion of noninsect taxa such as oligochaetes, gastropods, etc.

Table 2 lists the average densities (number per square metre) for the most common taxa (greater than 0.5% of the total density) that were collected from Padden Creek from June through October 1988. A complete listing of the 63 Padden Creek taxa is given in Hachmöller (1989). It should be noted that the des-

TABLE 2. Macroinvertebrate densities and nonmetric clustering (NMC) scores for major taxa.

Padden Creek macroinvertebrate taxa	% total density	Average densities (no./m^2)				NMC score
		Site 1	Site 2	Site 3	Site 4	
Plecoptera						
Malenka spp.	5.0	78.57	80.94	3.44	6.45	
Skwala spp.	0.6	3.87	11.84	1.72	2.15	0.47
Suwallia/Triznaka/Sweltsa complex	2.9	75.77	19.59	0.21	1.50	0.89
Ephemeroptera						
Baetis spp.	10.3	48.43	130.88	60.06	108.93	0.12
Cinygmula spp.	2.4	46.71	32.29	1.93	1.29	0.68
Epeorus spp.	3.9	95.58	33.58	1.29	0.21	
Ironodes spp.	2.3	36.38	32.50	4.73	3.01	0.47
Paraleptophlebia spp.	3.7	13.56	41.11	40.04	31.00	
Serratella spp.	1.9	2.36	57.04	4.52	1.50	
Trichoptera						
Glossosoma spp.	5.4	31.43	119.04	25.61	7.10	
Hydropsyche spp.	4.5	14.52	29.06	8.82	1.07	0.33
Rhyacophila spp.	1.1	24.54	10.33	1.29	0.64	0.89
Parapsyche spp.	4.8	8.18	12.70	107.63	33.15	−0.47
Diptera						
Chironomidae	7.7	60.70	87.83	59.20	54.03	0.26
Simuliidae	4.0	17.00	0.21	53.17	64.79	−0.33
Amphipoda						
Gammarus lacustris	0.7	0.00	0.86	5.59	17.43	−0.80
Annelida						
Enchytraeidae	31.4	210.97	260.05	237.88	355.42	−0.26
Lumbriculidae	2.2	3.44	9.25	23.03	39.61	−0.68
Gastropoda						
Ferissia	0.5	0.00	3.87	10.97	2.79	−0.41
Gyraulus	1.4	0.00	13.99	9.68	24.54	−0.26

ignation of "common" is somewhat arbitrary because, again, not all taxa were identified to the same level.

Confirmation of the observed longitudinal and seasonal trends by correspondence analysis can be seen in Fig. 2, which plots all samples by the first two components of correspondence analysis. Neither trend, however, corresponds well with a single component of correspondence analysis. Instead, the seasonal differences tend to spread along a "northwest–southeast" line, and the longitudinal trends spread along an orthogonal, north-east – southwest" line. We believe that this observation is important, as the emphasis in much statistical ecology is on recognizing a single, dominant gradient in the population. This is the motivation behind "detrended" correspondence analysis, for example, which attempts to force a one-dimensional ordination for data sets. In our case, a two-dimensional ordination was essential.

The ordinations by correspondence analysis led to difficulties in the identification of indicator taxa. First, as seen in Fig. 2, neither of the first two sample score components, alone, corresponds with the trends of interest. Each is a combination of both trends. Accordingly, neither of the first two taxa scores could be used to determine indicator taxa for either trend. Second, although correspondence analysis taxa scores were partially associated with the trends (for example, positive taxa scores were generally assigned to "upstream" taxa and negative taxa scores to "downstream" taxa) the correspondence analysis scores were strongly influenced by rare taxa. Only three of the top 20 correspondence analysis taxa scores were from common taxa, these three being *Hydropsyche*, *Malenka*, and *Serratella*.

The seasonal trend was confirmed by *k*-means clustering, which separated the samples by date. The June and July samples were placed in one cluster and the August, September, and October samples in the other, except for one August sample which was placed in with the June and July samples (see Fig. 2a). On the other hand, nonmetric clustering confirmed the observed longitudinal trend, and clustered all upstream (Sites 1 and 2) samples into one cluster and all downstream (Sites 3 and 4) samples into the other cluster (see Fig. 2b).

The attempt to identify indicator taxa using nonmetric clustering was very successful. Unlike correspondence analysis, most of the top taxa scores produced by nonmetric clustering were from common taxa. These 15 out of the 20 top scores were common taxa, and are listed in Table 2. This was impressive considering there were only 20 common taxa and that nonmetric clustering is "naive" in that it did not use total macroinvertebrate density as a selection criterion. Further, we verified the robustness of this taxonomic subset using a "leave-one-out" strategy. The nonmetric clustering taxa scores were recalculated based on only 19 "training" samples, leaving one sample out, and then the group (upstream or downstream) for the omitted sample was predicted using taxa scores generated from the other 19 samples. This procedure was repeated with each sample being the one omitted, obtaining 20 tests; thus we obtained an estimate of the rate at which errors might occur in using these taxa scores to classify unknown samples, by simply counting the number of the "left-out" samples that were mis-

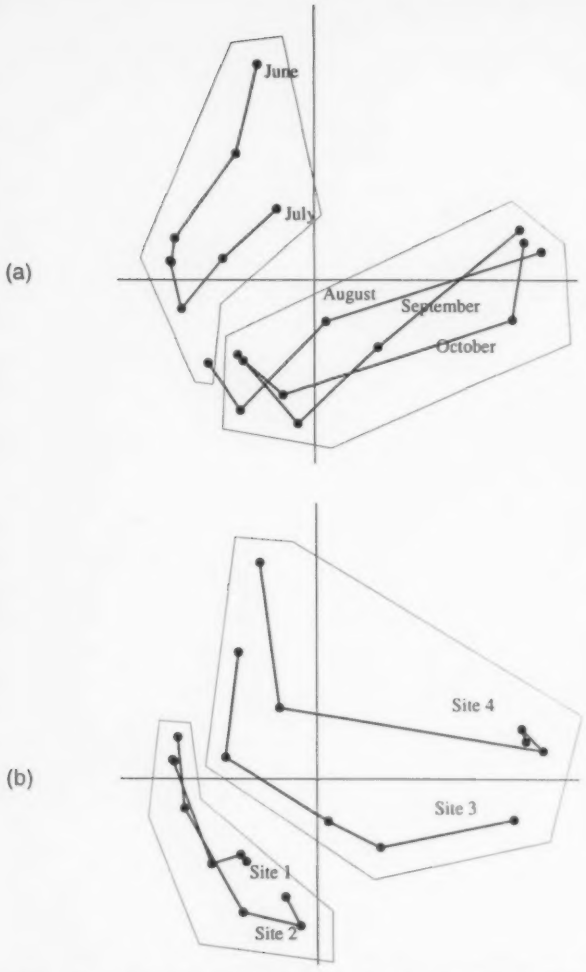

FIG. 2. Samples plotted with respect to the first two components of correspondence analysis. In Fig. 2a, heavy lines connect samples from a single date; in Fig. 2b, heavy lines connect samples from a single site. The "northwest–southeast" trend in dates and the "northeast–southwest" trend in sites are illustrated. Grouping of samples in Fig. 2a is by *k*-means clustering with squared Euclidean distance; grouping of samples in Fig. 2b is by nonmetric clustering.

classified. For our nonmetric clustering-derived characterization, there were no erroneous classifications. By comparison, we also performed "leave-one-out" testing using a linear discriminant procedure to reclassify the left-out sample. The linear discriminant misclassified 15% (3 out of 20), and this was in spite of the linear discriminant being an "informed" procedure, i.e. input to the linear discriminant procedure consisted of both the data points and an identification of which data points came from upstream samples and which from downstream samples. Nonmetric clustering is, in contrast, an "uninformed" procedure. Input to the nonmetric clustering procedure consisted only of the data points, and no information about the location of the samples. Nonmetric clustering was able to deduce the locations of the samples from the macroinvertebrate densities alone.

Discussion

Our statistical analyses supported our initial hypothesis that there were longitudinal and seasonal trends evident in the macroinvertebrate data. Ordination of samples by correspondence analysis was clearly possible (Fig. 2); however, a two-dimensional ordination was necessary to confirm each of the one-dimensional trends.

The existence of (at least) two gradients in a data set made interpretation of the data by clustering more difficult. Our two clustering techniques yielded radically different clusters because the structure of the data was complex enough to warrant two interpretations. Which trend is the "strongest" depends on how "strongest" is interpreted. In our professional judgement, the most obvious trend was the longitudinal trend. There were marked differences in the makeup of the macroinvertebrate communities from upstream and those from downstream. However, the existence of this "obvious" trend was not confirmed by *k*-means clustering. Instead, a rather new tool, nonmetric clustering, that approaches data clustering from radically different assumptions was required to "confirm the obvious."

The fact that correspondence analysis gave high scores to rare taxa might be expected because, if a taxon is rare, and only shows up at one site or date, it will, of course, be highly correlated with that site or data. But many factors can affect the reported densities of rare taxa, including drift and emergence as well as sampling technique, sorting, and taxonomic experience. Because only some of these factors are associated with a gradient, correspondence analysis may not be robust in data sets where there are many uncommon taxa. In Padden Creek, 43 of the 63 taxa were uncommon, i.e. making up less than 0.5% of the total density. The conclusion we draw is that taxa scores from correspondence analysis should not be viewed individually or in small subsets (such as the top 20), but only collectively.

Nonmetric clustering was the only technique that proved successful in both (a) confirming an observed trend and (b) providing a set of indicator taxa for that trend. Nonmetric clustering identified a subset of 15 common taxa, given in Table 2, that provided enough information to classify the samples, and did so more accurately than a linear discriminant.

Conclusion

Ecologically the dominant trends in our stream data were the longitudinal trend, where, typically, mayflies, stoneflies, and caddisflies were found at the upstream sites (Sites 1 and 2), while noninsects and tolerant taxa were found at the downstream sites (Sites 3 and 4), and the seasonal trend. Our subjective judgement was that the longitudinal trend was more significant in this study than the seasonal one. Correspondence analysis ordination of the macroinvertebrate data from Padden Creek confirmed the presence of both the longitudinal and seasonal trends in the taxa, but only as a "mixture" of each of the first two components of the ordination. In addition, correspondence analysis typically gave rare taxa the highest taxa scores, even though their relevance to large-scale trends in the data set was minor. *K*-means clustering favored the seasonal trend over the longitudinal trend, while nonmetric clustering favored the longitudinal trend. The nonmetric clustering also provided a robust means of simplifying the description of upstream and downstream clusters by identifying a set of 15 of the most com-

mon taxa that could be used to ordinate samples in other studies if a reduced sampling effort was desirable. This set of 15 proved to be a robust indicator of the location of the sample, regardless of the season in which the sample was collected. Nonmetric clustering has not previously been used to analyze benthic macroinvertebrate data, but should prove to be a useful tool for future studies, with broad applications and major advantages over current techniques.

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Appendix: Nonmetric Clustering

We give here a brief introduction to the technique of nonmetric clustering, which is described fully in (Matthews and Hearne 1991). Traditional clustering algorithms, such as *k*-means clustering, rely on a metric, or distance measure, defined over *n*-dimensional space. Points are then divided into clusters based on cluster "quality," where quality is in turn based on simultaneously minimizing intracluster distance and maximizing intercluster distance. In Fig. A.1, for instance, the points in the upper right would constitute one cluster because they are all close to each other, and the points in the lower left would constitute the second cluster because they are all close to each other and at the same time far from the points in the other cluster.

Problems arise with this method when other dimensions are added, however. In Fig. A.2a, the points all have the same x and y coordinates as in the previous figure, but a random value for the z dimension has been added. Intuitively, the points are still in the same clusters, and the third dimension represents pure noise that should be ignored. Metric-based clustering, however, must compose a metric out of all dimensions, with the result that the clusters proposed for the data are as shown in Fig. A.2b. If metric-based clustering is to succeed at all, some kind of data transformations or weighted metrics must be employed.

Nonmetric clustering, on the other hand, is not based on an *n*-dimensional metric. Instead, each dimension is examined independently of the others, and the association between the clustering and the dimension is measured. In Fig. A.1 the association between the obvious clusters and each of the x and y axes is evident. A quantitative measurement of this association is used to indicate the strength of the association. Guttman's λ (Goodman and Kruskal 1954), which is similar to a chi-squared statistic, is used for reasons discussed by Matthews and Hearne (1991). The optimal clustering, then, is selected as the one that has the strongest association with the largest number of dimensions. The dimensions themselves are not combined into a metric, and there is no call to include all dimensions in the estimate of clustering quality.

For our example data set, the nonmetric clustering for three dimensions, shown in Fig. A.2c is identical to the obviously "correct" clustering in two dimensions. This is because the best associations between clustering and dimensions are with the x and y axes. There is no way a clustering can be found that will associate well with more than two axes, and so only the x and y axes are used to measure clustering quality, and the z axis is ignored.

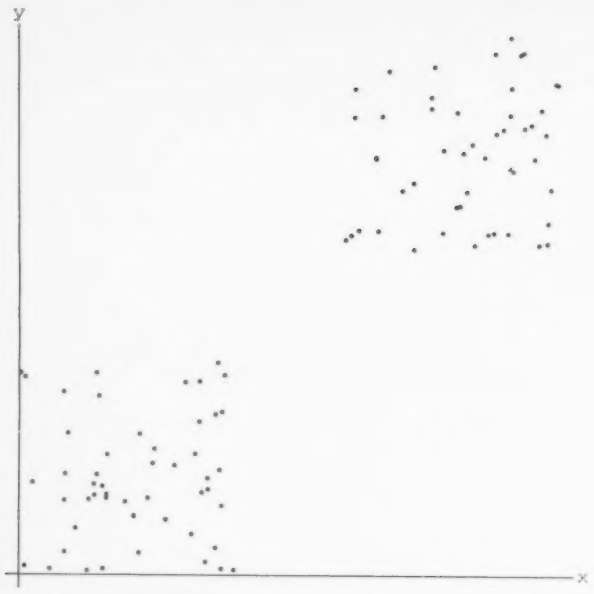

FIG. A.1. Artificially generated data set clustered in two dimensions.

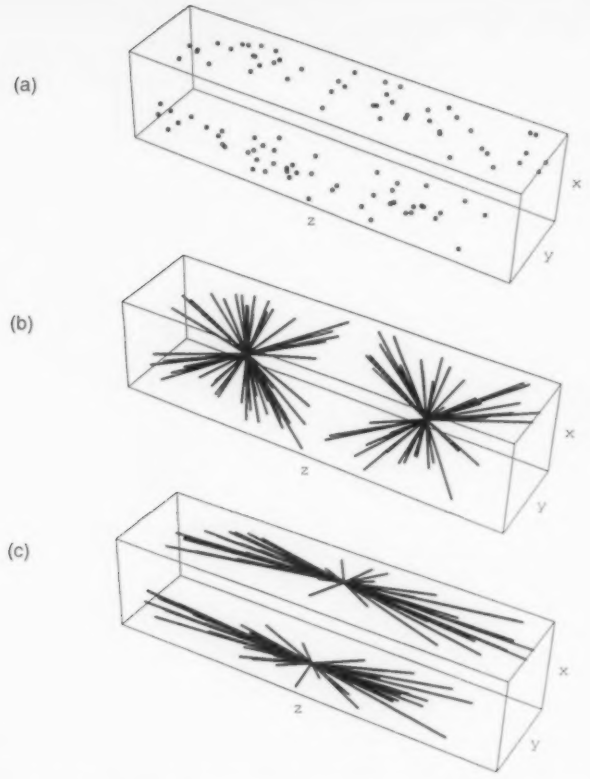

FIG. A.2. Artificial data set of Fig. A1 with (a) a random component in the z dimension added, (b) clustering by k-means, and (c) nonmetric clustering.

A computer program, called RIFFLE, implementing nonmetric clustering, has been constructed and is described in Matthews and Hearne (1991). RIFFLE was used for all nonmetric clustering discussed in this paper.

Nonmetric clustering thus offers the following advantages over traditional methods: (1) it does not combine counts from dissimilar taxa by means of sums of squares, or other ad hoc mathematical techniques; (2) it does not require transformations of the data, such as normalizing the variance; (3) it works without modification on incomplete data sets; (4) it can work without further assumptions on different data types (e.g. species counts or presence/absence data); (5) significance of a taxon to the analysis is not dependent on the absolute size of its count, so that taxa having a small total variance, such as rare taxa, can compete in importance with common taxa, and taxa with a large, random variance will not automatically be selected, to the exclusion of others; (6) it provides an integral measure of "how good" the clustering is, i.e. whether the data set differs from a random collection of points; and (7) it can, in some cases, identify a subset of the taxa that serve as reliable indicators of the physical environment; in our case, the indicator species were proved, in testing, to be more reliable than indicators based on a linear discriminant.

The primary disadvantages of nonmetric clustering, as we see them, are as follows. (1) There are some cases, documented in (Matthews and Hearne 1991), where metric clustering is to be preferred over nonmetric clustering. In general, we recommend using both, and examining the results critically, rather than accepting a single clustering method as the best for all cases. (2) The RIFFLE implementation of nonmetric clustering is very computer intensive, and takes much longer to run than k-means clustering. (3) Implementations of the technique, such as RIFFLE, are not widely available yet.

Systematics and Distributions of Dolly Varden (*Salvelinus malma*) and Bull Trout (*Salvelinus confluentus*) in North America

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Haas, G. R., and J. D. McPhail. 1991. Systematics and distributions of Dolly Varden (*Salvelinus malma*) and bull trout (*Salvelinus confluentus*) in North America. Can. J. Fish. Aquat. Sci. 48: 2191–2211.

Dolly Varden char in North America separate into two species: Dolly Varden (*Salvelinus malma* (Walbaum)) and bull trout (*Salvelinus confluentus* (Suckley)). Principal component analyses reveal two distinct morphotypes, and a linear discriminant function is derived (based on three field measureable variables) for their consistent classification. No single character can always distinguish both species, although branchiostegal ray number often will. The morphology of the two species remains relatively constant across their large ranges. The two species occur in strict sympatry in several areas with no evidence of interbreeding. The two species also exist parapatrically or syntopically and appear to exhibit broad scale competitive exclusion in these situations. Dolly Varden are distributed largely along the coast and range further north, while bull trout are mostly interior and range further south. Laboratory crosses demonstrate that the morphology of both species remain distinctive when they are reared under similar conditions and that their artificial hybrids are morphologically intermediate. No such natural hybrids were conclusively found, and there is no evidence of introgression.

En Amérique du Nord, le complexe Dolly Varden se divise en deux espèces : la Dolly Varden (*Salvelinus malma* (Walbaum)) et l'omble à tête plate (*Salvelinus confluentus* (Suckley)). Les résultats d'analyses des composantes principales révèlent deux morphotypes différents dont l'identification précise est fournie par une fonction discriminante linéaire basée sur trois variables qui peuvent être mesurées sur le terrain. Aucune caractéristique particulière ne permet d'identifier couramment les deux espèces, sauf, dans bon nombre de cas, le nombre de rayons branchiostèges. La morphologie des deux espèces demeure relativement constante dans leur vaste aire de répartition. Elles vivent en sympatrie étroite dans plusieurs régions, sans toutefois montrer de signes d'hybridation. Elles vivent aussi en parapatrie ou en syntrophie et semblent présenter une exclusion poussée en matière de compétition dans ces situations. La Dolly Varden fréquente dans une large mesure les eaux côtières et son aire de répartition se prolonge loin vers le nord, tandis que l'omble à tête plate fréquente surtout les eaux intérieures et son aire de répartition se prolonge loin vers le sud. Des croisements expérimentaux révèlent que la morphologie des deux espèces ne varie pas lorsque'elles sont élevées dans des conditions semblables et que leurs hybrides artificiels présentent une morphologie intermédiaire. Aucun hybride naturel du genre n'a été identifié avec certitude et il n'existe aucune preuve d'introgression.

Received December 3, 1990
Accepted May 1, 1991
(JA833)

Reçu le 3 décembre 1990
Accepté le 1 mai 1991

The salmonid genus *Salvelinus* has long been known as a difficult taxonomic group (Jordan et al. 1930; Berg 1948; Vladykov 1954), and in the north Pacific region this difficulty is exemplified by the Dolly Varden char (*S. malma*). Dolly Varden inhabit most drainages on both the North American and Asian coasts and have been recognized as a distinct species, at least in North America (Morton 1970; Armstrong and Morrow 1980; Behnke 1980, 1984, 1989; Cavender 1980; Morrow 1980) and Japan (Cavender and Kimura 1989; Kawanabe 1989), since their formal separation from the Arctic char (*S. alpinus*) species complex (DeLacy and Morton 1943; McPhail 1961). Some Soviet ichthyologists have also recently recognized Dolly Varden as a distinct species in Kamchatka (Glubokovsky and Chereshnev 1981; Chereshnev 1982; but see Savvaitova 1980a, 1980b, 1983) and some North American ichthyologists consistently have as well (Behnke 1980, 1984, 1989; Cavender 1980; Morrow 1980.) With species recognition, Dolly Varden acquired much of the notorious variability that was their part of the Arctic char species complex. Dolly Varden have consequently received considerable taxonomic attention as a distinct species complex (Morton 1970; Frohne 1973; Morrow 1973, 1980; Behnke 1980, 1984, 1989; McCart 1980) with their own similar difficult taxonomic problems.

An earlier analysis of Dolly Varden in North America by Cavender (1978) suggested that they consisted of two distinct species: Dolly Varden (*S. malma* (Walbaum)) and bull trout (*S. confluentus* (Suckley)). Although his study is persuasive, its results have not been universally recognized or applied. This is largely the consequence of shortcomings that are often typical of char systematic studies (McPhail 1961; Vladykov 1964; Mednikov et al. 1980; Savvaitova 1980a, 1980b.) His work is based solely on museum specimens, uses a typological species concept, does not incorporate ecological information, and inadequately analyzes only single characters and without statistical rigour. Furthermore, Cavender did not provide a field diagnosis that would consistently identify bull trout.

Individual characters rarely can completely separate species within char species complexes (McPhail 1961; Cavender 1978;

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Behnke 1980, 1984, 1989; Mednikov et al. 1980) and their proper identification often requires a multivariate statistical approach. Char populations can be characterized by a multivariate combination of variables that distinguish them from other populations (e.g. McPhail 1961; Ouellette and Qadri 1968; Frohne 1973; Henricson and Nyman 1976; McCart 1980; Morrow 1980; Dempson 1984; Mills 1989; Snorrason et al. 1989). However, the taxonomic significance of such statistical distinctions is uncertain without distributional and ecological information. Museum work is indispensable in char systematics because of the difficulty in obtaining enough specimens, but it is also often insufficient to decipher the considerable ecological variability in char (McPhail 1961; Savvaitova 1980b; Hammar 1984). The museum work should be supplemented by field studies and, if possible, by breeding experiments before decisions are made on taxonomic status.

Our study consists of several multivariate analyses of Dolly Varden throughout their definite range in North America. These analyses are interpreted using the criteria of coexistence in the biological species concept (Mayr 1963, 1969), the utility of which has been emphasized in char taxonomy (McPhail 1961; Savvaitova 1980a, 1980b, 1983; Chereshnev 1982; but see Behnke 1989). Although the biological species concept has some operational difficulties under certain circumstances (Ehrlich 1961; Hull 1970; Sokal and Crovello 1970; Wiley 1978), it is generally applicable in sexually reproducing sympatric populations (Paterson and McNamara 1984; Cracraft 1989). Under these conditions, the biological species concept is operational and also does not necessarily preclude other species concepts or engender particular mechanistic viewpoints (Coyne et al. 1988; Cracraft 1989; Masters and Spencer 1989; Templeton 1989). Since char are sexually reproducing and we have evidence of strict sympatry between Dolly Varden and bull trout, we use the biological species concept.

Materials and Methods

Study Approach and Outline

Regions of complete allopatry for Dolly Varden and bull trout in North America were identified from Cavender (1978) and verified in our study. These areas are the Queen Charlotte Islands in northwestern British Columbia (Canada) for Dolly Varden and the Kootenays in southeastern British Columbia for bull trout (see Fig. 7B.) Thirty museum specimens were selected from each allopatric region, and 93 measurements, 20 counts, and 23 truss measurements were made on each of the 60 char.

No single meristic or size-adjusted morphometric character completely distinguishes the two groups of 60 char, and this is concordant with Cavender (1978). Their validity as typological species was, therefore, multivariately tested using principal component analysis (PCA). After objectively establishing that the char from these two areas are morphologically distinct, a linear discriminant function (LDF) was derived that completely separates their two groups. This LDF is based on the multivariate combination of the minimum number of easily measured field characters that correctly classified all 60 specimens. These three LDF characters and an additional 23 truss measurements were then measured on all other collected or available specimens. From this material, the distribution of each species in North America was established, and possible areas of parapatry and strict sympatry were identified. These areas of coexistence were then sampled, and inter- and intraspecific crosses were reared under similar laboratory conditions, to determine if the two nominal char forms are good biological species or merely ecophenotypes.

Char Collections

The 1580 char (887 Dolly Varden and 693 bull trout) from 310 sample sites (149 for Dolly Varden and 161 for bull trout) are from five museums or new field collections now being deposited in the University of British Columbia Fish Museum. The samples cover all times of the year, and in some cases, multiple samples were available for the same sites from different periods and years. The five museums are Oregon State University (OSU), The California Academy of Sciences (CAS), University of Alberta (UA), University of British Columbia (UBC), and University of Washington (UW). The specific details for locality names and museum collections (using the above acronyms) are given in Fig. 7 and Appendix A.

Our field collections were made by gillnetting, electroshocking, seining, trapping, or angling. The char were preserved in 10% formalin until fixed and then placed in 37.5% isopropyl alcohol. Tissue samples of eye, heart, liver, and caudal muscle were also removed from each fresh specimen before preservation and frozen in liquid nitrogen at the field site. These tissue samples have not yet been analyzed.

Morphometrics and Meristics

The measurements and counts are explained in Appendix B and in Haas (1988). Linear measurements, conventional (Hubbs and Lagler 1964) or derived by us, are straight-line distances. The data set is complete with no missing values.

Body measurements were made with Helios vernier/dial calipers accurate to 0.10 cm. Where necessary, measurements and counts were made under a binocular dissecting microscope. As an accuracy check, all bilateral measurements and counts were made on both sides of the body, but by convention (Hubbs and Lagler 1964), only those from the left side were used in analyses. In consistent cases of strong bilateral asymmetry, the mean of the two counts or measurements was used or the two counts were added together. Accuracy was further verified by repeating all measurements and counts until the same or reasonably similar number was obtained twice. Wherever possible, counts were corroborated with available X-rayed and cleared/stained specimens. All data collection was by G.R.H. and is consistent throughout the study (Lee 1990).

The 23 truss measurements (Strauss and Bookstein 1982; Bookstein et al. 1985) were computed from 11 landmark points (Fig. 1). The landmark points were marked directly from the char specimens onto strong paper sheets and digitized. This simple and quick process allowed badly bent museum specimens to be properly measured and avoided problems of scale and parallax. The truss measurements for the 60 original char were also taken with calipers to verify our truss computer programs.

Measurement error was assessed by remeasuring, recounting, remarking, and redigitizing 10 specimens randomly chosen from the original 60 char (five from each of the two putative species). The mean amount of measurement error (Winans 1984) is negligible. A one-way ANOVA reveals that the ratio of individual to total (among plus within) variation is large (close to one), indicating that measurement repeatability is high and measurement error is insignificant (Falconer 1981; Baum-

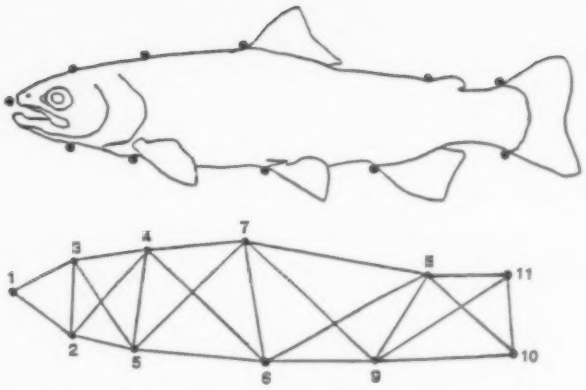


FIG. 1. Truss diagram (11 landmark points; 23 measurements). For definitions, see Appendix B and Haas (1988).

gartner et al. 1988). Each of the two groups also has similar patterns of insignificant error which avoids this potential complication in multivariate statistical analyses.

Statistical Analyses

The analyses are based on computer macros written within the S data analysis and graphics package (Becker and Chambers 1984). All our S programs were checked using previously published data sets and analyses and other similar programs available. The only exceptions are the one-way and two-way ANOVAs which were run on the pc-SAS statistical package and the two-way MANOVA which was run on mainframe computer SAS.

Univariate/bivariate allometric procedures

Morphometric characters were individually adjusted for body size both by divison with, and regression against, standard length (Haas 1988). This was only done prior to the preliminary univariate/bivariate allometric analyses. None of the meristic variables was size-confounded (Haas 1988) so they were directly compared (Reist 1985). Two common ratio methods were employed. The first is the straight division of each character for each individual by that individual's standard length (Reist 1985; Shea 1985) and the second is this same quotient but $\log_{10}$-transformed (Hills 1978; Reist 1985). Two standard least-squares regression techniques were also used. The first is necessarily based on $\log_{10}$-transformed data and uses the slopes derived from the regression to adjust the variables for each individual to an overall grand mean body size (Thorpe 1975; Reist 1985; Shea 1985; Claytor and MacCrimmon 1987). The second requires raw data, and the size-adjusted characters for each individual are taken as the residuals of this same, but untransformed, regression (Smith 1980; Reist 1985; Claytor and MacCrimmon 1987).

Standard length is used in all cases, since it correlates strongly with the other morphometric characters (Campbell 1976; Corruccini 1983) and otherwise removal of nonexistent size from them can result in false shape variables (Reist 1985; Somers 1986). Standard length is representative of "univariate size" (Mosimann and James 1979), and our use of total or caudal length produced identical results.

Character set reduction

It was necessary to reduce our complete original character set (116 measurements and 20 counts) for multivariate statistical analyses, since it contained more variables than specimens (60 char). Average-linkage cluster analyses on total covariance and correlation matrices (Jolliffe 1972, 1973; Thorpe 1976), done separately on the original morphometric and meristic data sets, were used to identify character combinations. Character groupings on scatter plots of the first two principal components of an R-mode PCA of total covariance and correlation matrices, done separately on the $\log_{10}$-transformed original morphometric and meristic data sets (Gould et al. 1974; Thorpe 1976), confirmed this dendogram character selection.

Characters were chosen from these clusters on the basis of previous knowledge (Cavender 1978), repeatability, ease of measurement, nonredundancy, usefulness, and interest (Thorpe 1976; Pimentel 1979; Bookstein et al. 1985). These characters were also selected to be strongly and positively correlated with each other, and in the case of the morphometric variables with size, since such data are best for multivariate allometric procedures (Campbell 1976; Corruccini 1983). In this way, the data set was reduced to the 51 morphometric characters used in the subsequent PCA and to the 10 meristic variables analyzed separately. These reduced variable sets are in Appendix B, with the complete original character set available in Haas (1988).

Ipsative measures in the reduced morphometric and meristic data sets were tested for independently through logical screening, matrix singularity checks (Pimentel 1979; Somers 1986), and by jackknifing (Neff and Marcus 1980; Gibson et al. 1984; Thorpe 1985). None were found.

Rao's paradox (Rao 1966; Healy 1969; Kowalski 1972) should not be a problem with our data (Corruccini 1987; Willig and Owen 1987). Our reduced morphometric character set has more statistically significant group differences based on a two-way MANOVA than on a one-way ANOVA, and better group separation is obtained in the multivariate case than in the univariate one (Haas 1988).

Reduced data set

The data used in our study are cross-sectional, and the size ranges and mean sizes of the two typological species are very similar. Any differences between the two groups are probably not based on size alone (Claytor and MacCrimmon 1987). These two putative species are also closely related and equivalently represented in our samples. Morphometric statistical procedures work best with such groups (Pimentel 1979).

The 30 char in each of the two original groups were chosen to represent similar broad size ranges, all typical habitats (lakes, rivers, and streams), and approximately equal numbers of both sexes. The Dolly Varden group is from seven separate localities and is composed of 15 males and 15 females, and the bull trout group is from 10 different sites and is composed of 11 males and 19 females. Two-way ANOVA (Thorpe 1976, 1980; Corruccini 1987) and two-way MANOVA done separately on morphometric and meristic characters (Thorpe 1976; Neff and Marcus 1980; Willig and Owen 1987) suggest that there is no significant univariate or multivariate sexual dimorphism in either group or in the total sample (p at least > 0.1). Furthermore, PCAs done separately on morphometric and meristic characters reveal no differences between males and females (Haas 1988).

Our sample of 30 for each of the two groups was used because a minimum sample size of 25 individuals is the "rule-of-

thumb'' often suggested for morphometric analyses (Neff and Marcus 1980; Reist 1985). Our sample size was later verified as adequate (Haas 1988) using both univariate and multivariate tests (Scagel et al. 1985; also see Cheverud et al. 1983).

Logarithmic transformation of morphometric data is almost always recommended (Bookstein et al. 1985; Shea 1985; Bryant 1986) and was undertaken here. A square-root transformation is sometimes suggested for meristic data (Sokal 1965; Pimentel 1979), but with our data this produced similar results to the logarithmic transformation (Haas 1988), so only logarithmic transformation was used.

The statistical assumptions of our multivariate morphometric procedures for our data were tested (Haas 1988). Our morphometric and meristic data are normally distributed under both univariate and multivariate conditions when they are $\log_{10}$-transformed. Our univariate normality test was the probability (quantile) plot correlation coefficient procedure (Ryan and Joiner 1974; Filliben 1975; Ryan et al. 1976). Our multivariate normality test was also based on probability plots and allowed for a visual assessment of whether the probability plot approximated a straight line which would represent multivariate normality (Healey 1968; Wilk and Gnanadesikan 1968; Wilk and Shapiro 1968; Andrews et al. 1973; Everitt 1978). As well, multivariate normality was evaluated through PCA scatter plots which tend to have an ellipsoidal distribution (Fig. 2 and 3) if the data they are based on are multivariately normally distributed (Thorpe 1976; Reyment et al. 1984).

Homoscedasticity was conservatively tested (Phillips et al. 1973; Somers 1986) using Box's modification of Bartlett's test (Pimentel 1979). Our $\log_{10}$-transformed morphometric and meristic data are homoscedastic ($p > 0.5$) for the two putative species and for both sexes. This enabled us to use a total data matrix rather than a pooled within-group matrix for the subsequent multivariate analyses (Phillips et al. 1973; Pimentel 1979; Somers 1986). The individual character variances and their linearity are also greatly standardized by $\log_{10}$-transformation (Haas 1988).

Multivariate allometric procedure — Principal component analysis (PCA)

PCA is used to objectively verify and define the two typological species assumed for the 60 original char. Q-mode PCA was independently performed on total covariance matrices of the $\log_{10}$-transformed reduced character set of 51 morphometric variables and on the $\log_{10}$-transformed set of 23 truss measurements. This PCA procedure was used because when it was compared with PCA done on a total correlation matrix, with sheared PCA (Humphries et al. 1981; Bookstein et al. 1985), and with Burnaby's PCA method (Burnaby 1966; Rohlf and Bookstein 1987; Baumgartner et al. 1988), it produced virtually identical group ordination yet more realistic and representative character and individual data information (Pimentel 1979; Corruccini 1983; Haas 1988). The overall similarity between these PCA techniques suggests that our PCA is realistic. The PCA ordination and loadings were later verified over the entire sample of 1580 specimens and the sample of laboratory crosses using the $\log_{10}$-transformed 23 truss measurements in both cases.

Only the first two eigenvectors and principal components are significant and were analyzed. Significance was tested using Bartlett's chi-square test of sphericity (Phillips et al. 1973; Pimentel 1979) and the graphical Scree test (Cattell 1966; Somers 1986).

This same PCA was carried out separately on the reduced set of 10 meristic characters and comparable results were achieved (Haas 1988). Since the meristic variables are not size-confounded, this meristic PCA did not provide easily interpretable size/shape information and was used only as a check on the morphometric test (Somers 1986).

Multivariate procedure — Linear discriminant function analysis (LDFA)

LDFA with equal group assignment probabilities was used to create an unweighted LDF that completely separates the two typological species present in the 60 original char on the first discriminant function (100% correct classification, 0% error rate). A jackknife procedure based on individuals was later used in conjunction with the LDFA to verify the classification and error rates. As well, the LDFA was checked over our entire data set and subsets of it to ensure that the LDF derived from the original 60 char worked for all samples sites and their combinations.

The LDF is based on branchiostegal ray number, anal fin ray number, and the ratio of total upper jaw length to standard length. These four variables constitute a minimum set sufficient for complete group separation. They also load strongly on and have good correlation/percentage variance patterns (Pimentel 1979) with the second PCA eigenvector (shape information) based separately on the reduced morphometric and meristic data sets (Haas 1988). As well, these three characters on their own partially separate the two putative species, and all can be made in the field without killing fish. Division by standard length was used to crudely, but easily and sufficiently (for this LDFA), adjust total upper jaw length for body size.

The overall similarity between our PCA and LDFA results lends more support to the conclusions of these analyses (Claytor and MacCrimmon 1987). This similarity also further justifies the use of a total unpooled data matrix for the PCA, since LDFA accounting for the groups did not change the outcome (Campbell 1976; Bookstein et al. 1985; Shea 1985).

Laboratory Crosses

Inter- and intraspecific laboratory crosses were made between Dolly Varden and bull trout collected from separate, but nearby, locations in the lower Fraser River system, British Columbia, Canada (Appendix A and Fig. 7B). The Dolly Varden are from sample site 26 (Katherine Lake; 49°20′50″N, 122°33′0″W) and the bull trout from sites 181 and 182 (Foley Creek/Lake; 49°7′35″N, 121°35′10″W).

The char were collected in November as migrating spawners by electroshocking and transported alive to the laboratory. The two types were separately kept in an large outdoor concrete pond (2.5 by 8 m in total) evenly divided into two long sections. This pond had a natural dechlorinated stream flow (8°C) and pea gravel/small rock covered bottom. The char were fed commercial hatchery fish food until they were in final spawning condition. This was marked by their attempts to build redds and spawn in the concrete pond.

Eggs were then collected from individual females and divided into two equal lots. Each lot for each female was crossed with either a Dolly Varden or bull trout male. Eight sets of these crosses were made (four Dolly Varden females and four bull trout female), and the fertilized egg lots were then separately placed in an incubation chamber and slowly adjusted to a temperature of 4°C (McPhail and Murray 1979; Brown 1985; Carl

1985). The adults were killed, preserved, and measured as described previously.

The incubation chamber consisted of a single water-filled refrigeration unit (Murray and McPhail 1988) that maintained the 4°C temperature and darkness (except for brief daily examination periods). The individual incubation containers for each egg lot in this refrigerator system were aquarium filter boxes which had bottoms covered with pea gravel and had porous lids supplemented with fine-screen material to exclude foreign material and retain the alevins after hatching. Each filter box had its own air supply. The initial rearing densities were similar and even after dead egg removal they remained comparable throughout the experiment. Dead eggs were preserved in Stockard's solution.

Hatching of the crosses occurred at roughly the same time over a 4- to 5-d period. Incubation time at the 4°C temperature was about 85 d. After the eggs hatched, the fish were transferred to aerated buckets of 4°C water and allowed to acclimate to the natural water supply temperature (8°C). They were then put in a flow-through fibreglass rearing trough (0.8 by 2.9 m) divided into 16 equal segments which was located outside under a natural light and air temperature regime.

The char were initially fed live brine shrimp nauplii and ground Tetramin tropical fish food several times daily. They later received commercial fish pellets, frozen brine shrimp, finely chopped frozen liver, tubifex, and other available live food on a daily or twice-daily basis. Feeding was to at least saturation, and all excess food and waste material was syphoned off daily. All dead fry were removed. Mortality was unbiased and low, so rearing densities remained comparable between the crosses.

Both the parental and hybrid char developed normally, but a strong chlorine pulse in the water system killed all the fish at a small size (mean standard length = 5.16 cm). All char were kept, preserved, and measured as discussed previously.

The LDF and truss measurements were taken on 90 laboratory cross fish. These 90 char comprised 30 pure Dolly Varden, 30 pure bull trout, and 30 hybrids (15 from each of the two reciprocal hybrid possibilities.) A Q-mode PCA on a total covariance matrix of the $\log_{10}$-transformed truss measurements and the LDF derived from the entire data set were used to see if the three types could be distinguished, if the results were comparable with our previous analyses, and if there were hybrids in the natural samples. Our LDFA classifies only the two putative species, so we did not attempt to directly discriminate known hybrids with this method.

Results

PCAs

The PCAs on the original 60 char based on the reduced 51 morphometric character data set (Fig. 2A) and on the 23 truss measurements (Fig. 2B) similarly reveal that Dolly Varden and bull trout are clearly distinct. The group centroids show the same pattern. The centroids and scatter points also respectively indicate that the analyzed size means and ranges of the two forms are very similar.

In both cases, this group distinction is based solely on the second principal component (PC) which is interpreted here and traditionally as a "shape information" component statistically independent (orthogonal) of size variation (Jolicoeur 1963; Bookstein 1989). The first PC is customarily deemed a "size information" component.

Both these classic PCA features of "size" and "shape" are present in our data (Haas 1988). The first eigenvectors used to calculate our first PCs are general (all loadings have the same sign) and account for the vast majority of the variability (large eigenvalues, see Fig. 2). Their general signs are indicative of a single-type of variation (Pimentel 1979) and their large eigenvalues make intuitive sense, since size-related variation should predominate in our data sets. These are the typical features of a size component and suggest that allometric size has been effectively removed in the first PC (Campbell 1976; Reyment et al. 1984).

Our second eigenvectors are bipolar (loadings have mixed signs) and account for all the remaining statistically significant variation (Bartlett's test, $p < 0.05$; Scree test). These are typical aspects of a shape component. Further support for our size and shape interpretations is that char size and weight are strongly correlated with PC1 and uncorrelated with PC2. In both cases, large char have higher scores on PC1 whereas PC2 does not distinguish between these measures of fish size but rather between the two groups.

The distinction between Dolly Varden and bull trout in Fig. 2 also shows that the species' groupings are better separated by the larger reduced data set in Fig. 2A, but that the group differences using the truss measurements in Fig. 2B are similar overall and identical in terms of their group centroids. This clear and similar delineation of both the individuals and their group means also occurs in the single PCA based on the 23 truss measurements collected on the entire 1580 char in our data set (Fig. 3). While the distinction in Fig. 3 is perhaps not as definite overall, this PCA is analyzing these char from across their North American ranges. If the individuals plotted on Fig. 3 are analyzed from smaller geographic regions or sample sites, their group designations are again definite. Furthermore, the group centroids for our total data set continue to remain defined (Fig. 3D). The group centroids and the plots themselves also demonstrate that similar mean sizes and ranges were analyzed for each species in our total data set.

Individual LDF Characters and Their LDFA

The three individual characters used to construct the LDF for distinguishing Dolly Varden and bull trout can themselves each partially differentiate these two species on a statistically significant basis (Fig. 4A–4C). The best single character is branchiostegal ray number (Fig. 4A). This trait alone would distinguish the two species in a large number of situations. The majority of Dolly Varden have a range of branchiostegal ray counts from 17 to 23 with a few individuals in the 24–25 range, whereas most bull trout have counts of 26–31 with a few individuals in the (22)23–25 region. The median branchiostegal ray count for Dolly Varden is 22 and for bull trout is 27.

Anal fin ray number (Fig. 4B) and the ratio of upper jaw length to standard length (Fig. 4C) are not as effective in group separation as branchiostegal ray number. Anal fin ray number for bull trout does show a strong median of 12 whereas that for Dolly Varden is around 11. This canalization of anal fin ray number for bull trout is pronounced enough that all other anal fin ray counts obtained for them are plotted as statistical outliers. Bull trout also have larger upper jaws in proportion to their body lengths than Dolly Varden. This latter measurement may be more variable or difficult to take, since the number of outlying points is higher than for the other two measures.

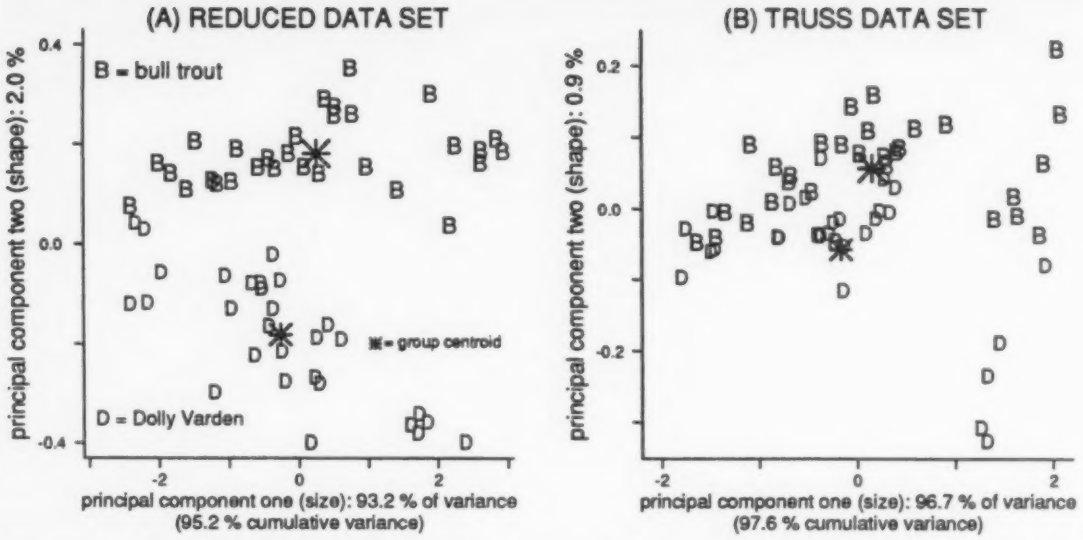

(A) REDUCED DATA SET
B = bull trout
D = Dolly Varden
* = group centroid
principal component two (shape): 2.0 %
principal component one (size): 93.2 % of variance
(95.2 % cumulative variance)
(B) TRUSS DATA SET
principal component two (shape): 0.9 %
principal component one (size): 96.7 % of variance
(97.6 % cumulative variance)

FIG. 2. Scatter plots of the PCAs done on the original 60 char (30 specimens of each species from a respective region of complete allopatry for both) based on (A) the reduced 51 morphometric character set and (B) the 23 measurement truss data set.

LDFA combines the discriminating power of these three characters into a single measure that consistently identifies the two species. The LDFA shown in Fig. 4D clearly discriminates between Dolly Varden and bull trout on the first LDF for every fish. This LDFA worked for covariance or correlation matrices on raw or $\log_{10}$-transformed data. The LDF presented below is based on a covariance matrix of untransformed measurements for our entire data set of 1580 fish. It was chosen since the analytical outcomes are the same, raw measurements are the easiest to use, and the LDF formula is most refined for our complete data set.

The LDF formula based on untransformed data is

$$0.629 \times \text{branchiostegal ray number} + 0.178 \times \text{anal fin ray number} + 37.310 \times \text{total upper jaw length/standard length} - 21.8.$$

The interpretation of this LDF formula is that char > 0 are bull trout and < 0 are Dolly Varden.

Laboratory Crosses

The PCA of the 23 measurement truss data set for the two species and their reciprocal hybrids clearly separates these three groups and their centroids (Fig. 5A). The Dolly Varden and bull trout are ordinated as in the natural samples (Fig. 2 and 3), and their hybrids appear as intermediates. This separation of groups is again based only on the second PC shape axis and is unconfounded by size. This size/shape interpretation here is the same as was previously substantiated in the PCA results section.

Somewhat less variation is accounted for in the first PC in the laboratory cross PCA (Fig. 5A) than for the previous PCAs (Fig. 2 and 3). Also, PCs subsequent to the second contain statistically significant information in the laboratory cross PCA. This is likely because the fish in the laboratory crosses were all small and of similar size. With less size variation in the data, the variation accounted for in the PCA could be spread across more PCs. This has litle effect on our standard interpretation of the first two PCs as shown in Fig. 5A, and this analysis has direct correspondence to our previous PCAs.

The slight size variation that does appear in the laboratory cross fish demonstrates that bull trout appear to grow somewhat faster than Dolly Varden, at least to this small size. Their hybrids are intermediate in growth rate. This can be seen both by the general scatter points and by the group centroids. However, the bull trout used in the crosses were slightly larger than the Dolly Varden used, and this size difference may be confounding these apparent growth differences.

The LDF derived from our entire data set discriminates the three laboratory cross groups in the same manner (Fig. 5B) as the PCA and as the previous LDF (Fig. 4D). This discrimination is again statistically significant for all three groups. Dolly Varden and bull trout continue to be easily separated at the 0 discriminant level of the first LDF (Fig. 4D), and the hybrids occupy an intermediate discriminant space with their median falling near this 0 value (Fig. 5B). This hybrid discriminant space is well outside the majority of pure individuals in Fig. 5B and 4D. Although the hybrids are intermediate, they are somewhat closer in position to the Dolly Varden in both of these figures. However, the overall degree of LDF data overlap is higher with bull trout.

Discussion

Species Identification

The existence of two distinct typological species within Dolly Varden has been objectively demonstrated using PCAs of several different data sets and combinations of them. Furthermore, the characters that distinguish the two species are relatively consistent throughout their broad ranges as studied herein. Both species also remain distinct when reared under similar laboratory conditions. A working diagnostic distinction between the two species can be consistently made using an LDF formula based on branchiostegal ray number, anal fin ray number, and the ratio of total upper jaw length to standard

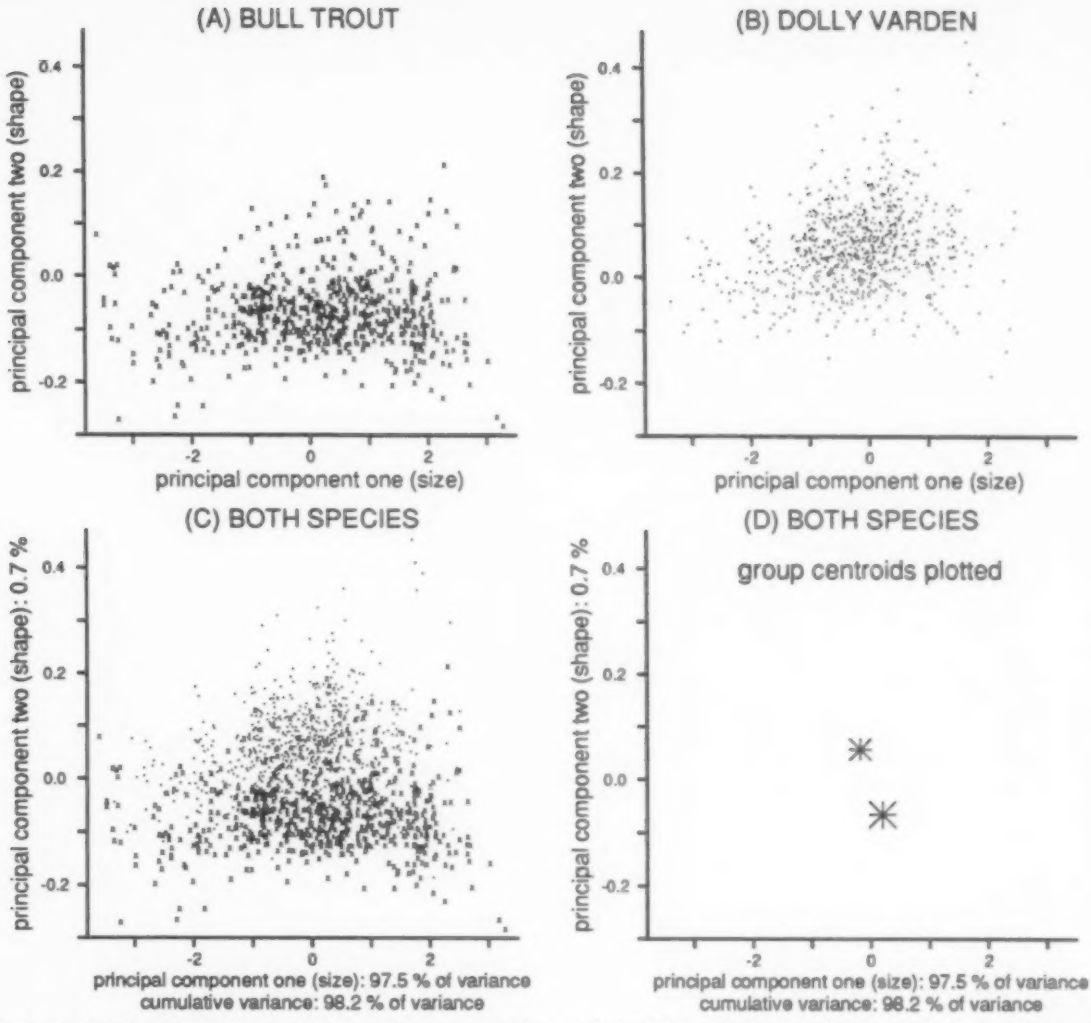


FIG. 3. Scatter plot of the PCA done on the entire 1580 char in our data set. This PCA is a single analysis based on the 23 truss measurements and is only plotted in four sections for clarity of graphic presentation.

length. Each of these three individual measurements can themselves distinguish the two species in some geographic areas, but only their combined discriminating power in the LDF formula enables consistent separation of Dolly Varden and bull trout. Our study corroborates Cavender's (1978) assertion that North American Dolly Varden are separable into two typological species: Dolly Varden and bull trout (Fig. 6).

Cavender (1978, 1980; also see Medvedeva and Savvaitova 1980) presented additional differences between Dolly Varden and bull trout in skull osteology and gill raker morphology. Gould (1978) examined bull trout development from the egg stage to yok sac absorption and found several minor, and one unique, difference from similar information published for Dolly Varden (Blackett 1968; Armstrong and Blackett 1980). He emphasized that these ontogenetic differences, especially his gular ridge character unique to bull trout, are as large as those developmental differences between other char species.

Cavender (1984), Cavender and Kimura (1989), and Phillips et al. (1989a, 1989b) demonstrated cytological differences between Dolly Varden and bull trout in their ploidy, karyotype arrangement and chromosomal organizer regions. As well, Grewe et al. (1990) showed evidence of their mitochondrial DNA divergence. This cytological and genetic work also indicates that these two char species are not sister taxa, with Dolly Varden being more closely related to Arctic char and with bull trout being more closely related to the white spotted char (*S. leucomaenis*) of Asia (also see Behnke 1989). These phylogenetic relationships are further evidence that Dolly Varden and bull trout are not simply ecophenotypes of the same char species.

Qualitative features for the identification of Dolly Varden and bull trout have been suggested (e.g. Cavender 1978; Goetz 1989), but none that we know of work consistently throughout their range. Some may work over smaller geographic areas and can perhaps be investigated after proper species identification with our LDF. Also, LDFs based on our three characters can probably be derived that function even better for certain regions than our overall LDF. For many situations, total branchiostegal

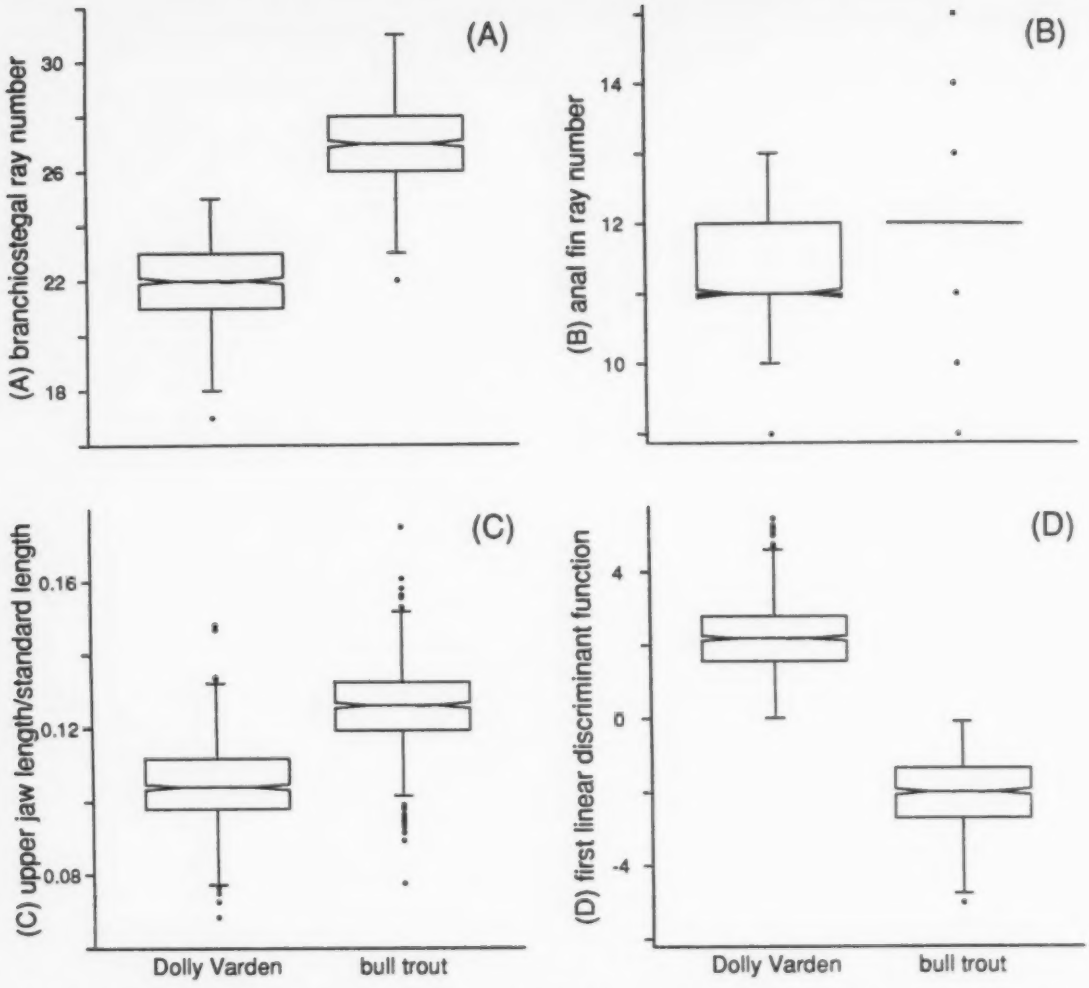


FIG. 4. Boxplots of (A–C) the three best discriminating characters and (D) their multivariate combination. Each set is for all the 1580 specimens in our data. The horizontal line drawn through each box is each species' data median, and the vertical lines extending from them encompass their respective data ranges with statistical outliers being plotted as separate points. Nonoverlapping notches between each species box in each set indicate a statistical difference at a rough 5% significance level (Chambers et al. 1983).

ray number is sufficient to identify the two species, but where this is not the case the LDF formula should be used. If pressed for a qualitative expression of the two species' forms, we could generally say that bull trout have larger, broader, and flatter heads and their bodies are more slender and ventrally flattened. Dolly Varden have more oval and "snake-like" bodies with their heads not dominating their profile (Fig. 6).

Hybrids, Sympatry, and Parapatry

The PCA of and LDF for the laboratory crosses indicate that hybrids are morphologically intermediate to Dolly Varden and bull trout. The PCA and LDF results from our entire data set suggest that such hybrids are nonexistent, or at least infrequent, in nature. Very few wild fish of either species occupy this intermediate hybrid morphological space, especially when the data are analyzed by site or region. If hybrids occur naturally, they are not common and there certainly is no evidence here of introgression between the two species (Cavender 1978).

Cavender (1978) suggested that two specimens from two lakes in the Skeena River drainage in British Columbia might be hybrids. However, his hybrid analysis is based on a qualitative comparison of four individual characters, two of which we found to be of little overall taxonomic value. Our PCAs and LDF could perhaps have properly classified his suspected hybrids, but the specimens could not be verified, since the museum samples and lakes they came from were not readily accessible to us.

Possible introgressed populations in the Skagit River region of Washington have been independently identified (K. Kramer, Washington Department of Game, 16018 Millcreek Blvd., Millcreek, WA 98012, USA, pers. comm.) since this paper first went to press. These identifications are based on our older LDF (Haas 1988) but have not yet been verified by us and we did not analyze this region in detail because of a lack of specimens and collecting access. We do not feel this affects our species' designations but do want to bring this interesting pos-

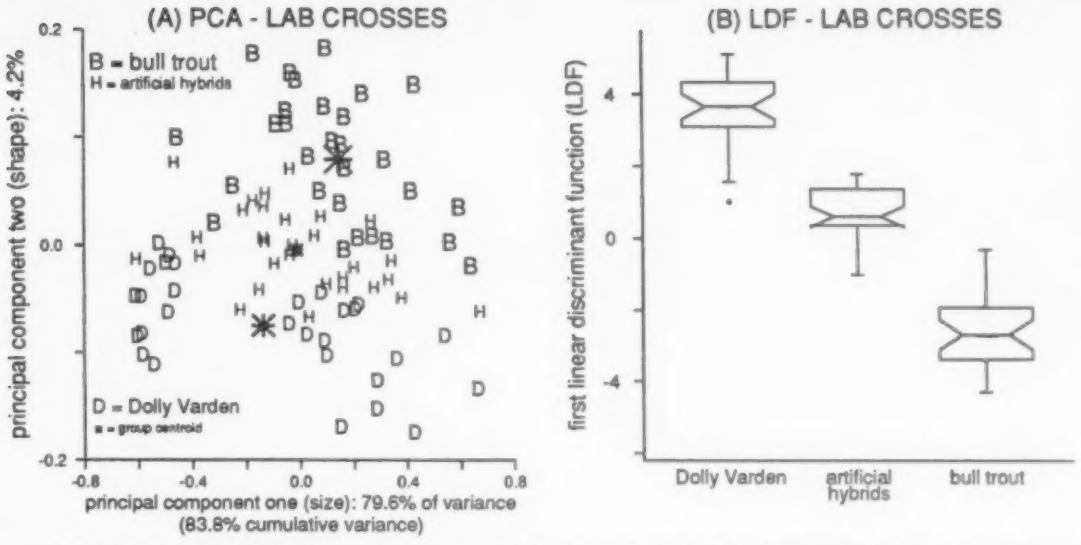


FIG. 5. (A) PCA and (B) LDF of the laboratory-reared species and their reciprocal artificial hybrids. Figure 5A is based on the 23 measurement truss data set and Fig. 5B on the LDF derived from the total data set using the same three previous LDF characters. In Fig. 5B, the horizontal line in each box is each species' data median, and the vertical lines extending from them encompass their respective data ranges with the statistical outlier being plotted as a separate point. Nonoverlapping notches between each species box in each set indicate a statistical difference at a rough 5% significance level (Chambers et al. 1983).

sibility to attention. We did not find introgression or hybridization elsewhere; these possible Skagit River region introgressions are only occurring in one small area at the southern range limit of Dolly Varden (see following sections on geographic distributions), and there is the other aforementioned evidence supporting our conclusions on species' status.

We found Dolly Varden and bull trout in strict sympatry, with no evidence of interbreeding, at 10 sample sites in four river drainages. Thus, Dolly Varden and bull trout are not only typological species but good biological species in the strictest sense (Mayr 1963, 1969). Dolly Varden predominate in these sympatric samples, and this suggests that they may somehow do better in strict sympatric situations. Bull trout and introduced eastern brook trout (*S. fontinalis*) coexist and even hybridize in several areas (Paetz and Nelson 1970; Cavender 1978; Behnke 1980; Leary et al. 1983, 1985; Goetz 1989; G. R. Haas and J. D. McPhail, unpubl. data), and bull trout also appear to do less well in these localities (Leary et al. 1983; Goetz 1989; D. Markle, Department of Fisheries and Wildlife, Oregon State University, Corvallis, OR 97331, USA, unpubl. data; W. Roberts, Department of Zoology, University of Alberta, Edmonton, Alta. T6G 2E9, Canada, pers. comm.; M. Rode, California Department of Fish and Game, 3 N. Old Stage Rd., Mount Shasta, CA 96067, USA, unpubl. data). On the other hand, Dolly Varden do tend to be more abundant even in allopatric samples than bull trout (see char collections in Materials and Methods section), and Dolly Varden may simply be more numerous in general.

By strict sympatry, we mean samples that were collected in the same location at the same time or museum specimens similarly sampled and placed in the same museum collection. There were field samples and museum collections of the two species from the same sites that were not classified as strictly sympatric because they were sampled independently or were in more than one museum or museum collection. Also, some samples in regions of potential sympatry contained only one specimen, so sympatry could not be assessed. Some of these sites may actually be strictly sympatric if they are sampled further.

The four river drainages containing strict sympatric samples are the lower Fraser River, Skeena River, Nass River, and Stikine River, all in British Columbia (Fig. 7B). This is similar to Cavender's (1978) findings. Cavender also suggested that sympatry exists in the Puget Sound region of Washington and in the drainage areas of northwestern British Columbia, southeastern Alaska, and southwestern Yukon. We could not verify this, but our data indicate that it is possible. All 10 of our strict sympatric sites make sense in terms of the zoogeography of Dolly Varden and bull trout (see following section on geographic distributions) except for sample 2. This sample is from the upper Fraser River drainage area and all our other samples from this area are bull trout. We cannot explain the Dolly Varden in this sympatric sample and assume it is a museum error.

Many additional populations of Dolly Varden and bull trout exist under parapatric or syntopic conditions (Fig. 7A and 7B). In watersheds where both species occur we often find only one species at a site, but adjacent systems within that watershed can vary in which species it is. As well, the two species often separate in single drainages on the basis of lake and stream habitats or headwater and lower river reaches (Cavender 1978). Neither of the species appears to dominate in any of these situations and this suggests competitive exclusion. No strong evidence of character displacement is readily apparent in our analyses and any ecological interactions between the species may generally result in broad-scale exclusion rather than in fine-scale resource partitioning. Sympatry does still occur but it may be short term or restricted to narrower areas with particular conditions.

The char used in our laboratory crosses are from separate but nearby sites in the lower Fraser River drainage in British Columbia. In this general area the species occur both in parapatric or syntopic conditions and in sympatry. The Dolly Varden used

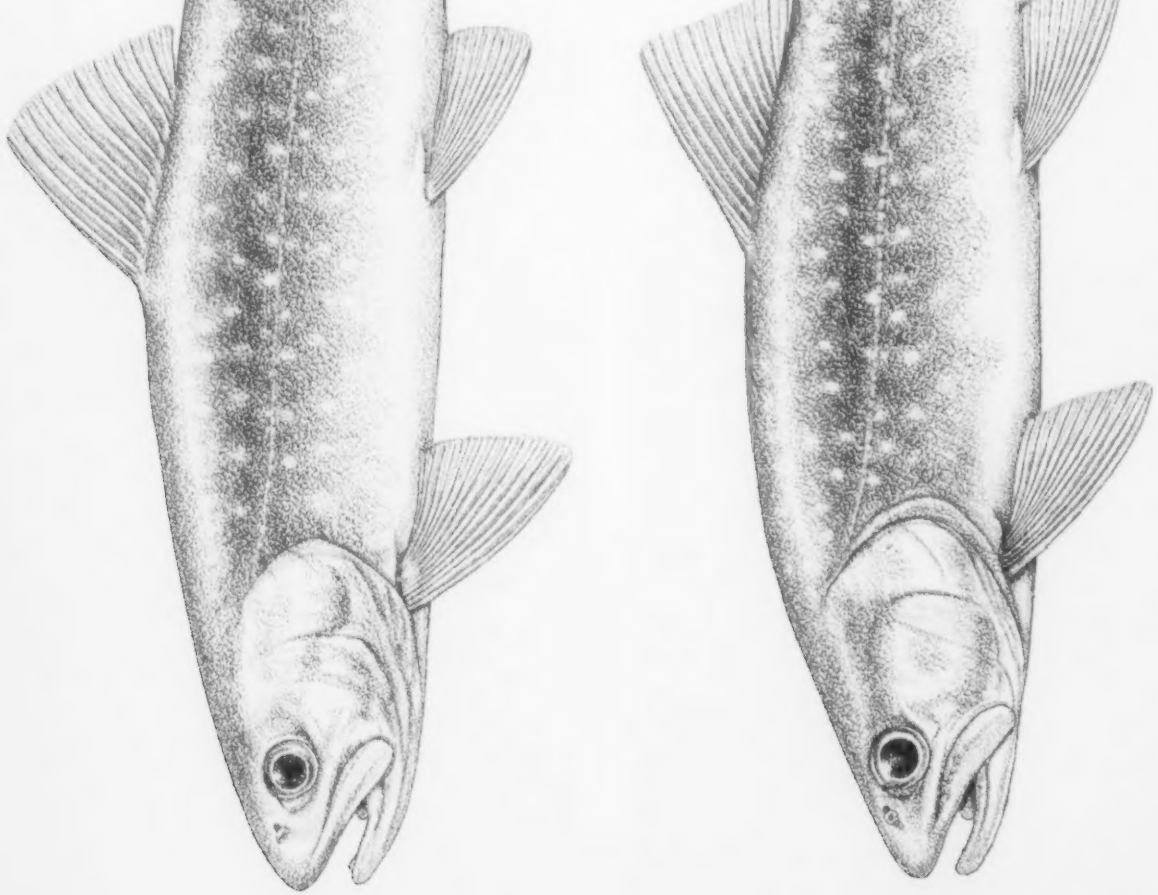
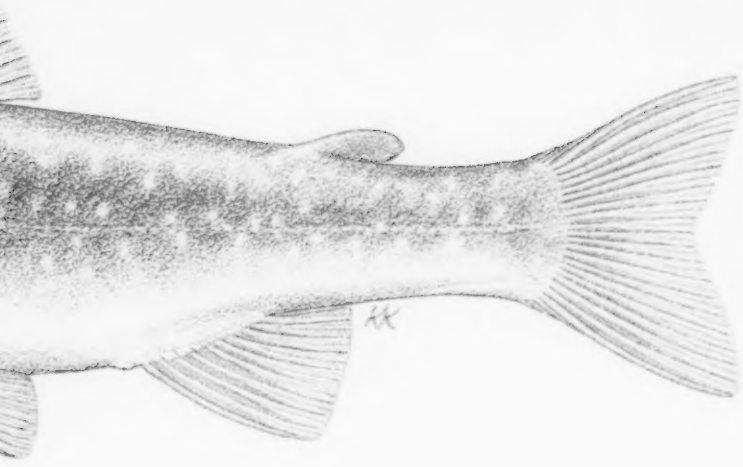
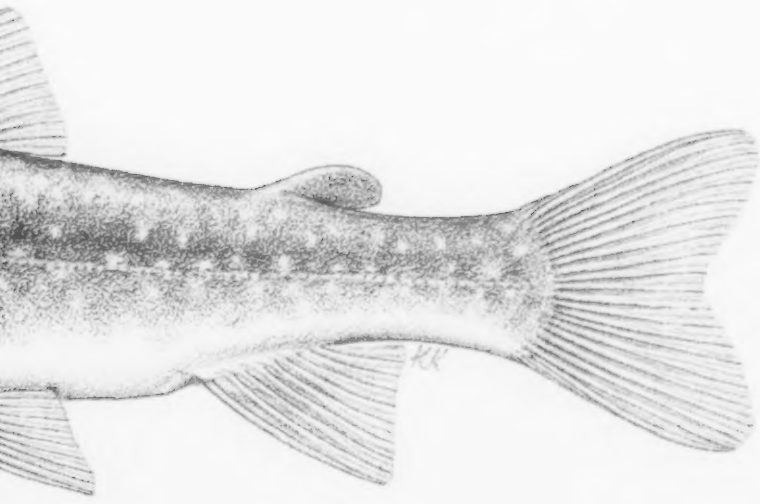


FIG. 6. Composite drawings of the two char species. The top drawing is a Dolly Varden and the





and the bottom is a bull trout. The drawings were done for us by Karen Klitz.

in our crosses developed spectacular, sexually dimorphic spawning characteristics and colouration whereas the bull trout used did not possess any typical char spawning characteristics or colouration (colour photographs in Haas 1988). In contrast, bull trout in allopatric areas show typical char spawning characters and colours (McPhail and Murray 1979; Leggett 1980; G. R. Haas, pers. obs.). This suggests the possibility of some reproductive interactions between the two species in systems where they coexist, even if that coexistence may not be strictly sympatric.

Leggett (1980) described the spawning behaviour of allopatric bull trout but was unaware of their taxonomic distinctiveness. Nevertheless, he noted behavioural differences between his interior British Columbia population (also see Needham and Vaughan 1952) and information published for allopatric Dolly Varden populations from coastal Alaska (Blackett 1968). The differences in spawning behaviour between these same populations were also noticed by Armstrong and Morrow (1980) and more directly linked to the Dolly Varden/bull trout distinction. Spawning behaviour in sympatric or parapatric/syntopic drainages has not been assessed.

Nomenclature

Our analysis of the nomenclature and taxonomic history of these two species was limited to a literature review. We were unable to examine the type specimens of Dolly Varden, bull trout, or any of their potential synonyms due to their museum storage localities and unsatisfactory preservation states. We therefore tentatively agree with Cavender (1978) on the scientific details of naming bull trout, but our opinion could change upon examination of type specimens. Inspection of char specimens from Asia and from more northern regions could also affect this nomenclature.

The nomenclatorial history of Dolly Varden is described in DeLacy and Morton (1943), McPhail (1961), McPhail and Lindsey (1970), Morton (1970), and Scott and Crossman (1973). The nomenclature, taxonomic history, and diagnostic description of bull trout are available in Cavender (1978). To the best of our present knowledge, the appropriate names (Cavender 1978) for the two species are Dolly Varden (*Salvelinus malma* (Walbaum)) and bull trout (*Salvelinus confluentus* (Suckley)).

Geographic Distributions

Dolly Varden (S. malma)

Dolly Varden range from just south of the Canada–US border (≈47°N) to the Seward Peninsula (≈60–62°N) in Alaska (Fig. 7). Beyond this northern limit, most char appear to be forms of so-called western Arctic char (McPhail 1961; McPhail and Lindsey 1970; Johnson 1980; McCart 1980) or northern Dolly Varden (Behnke 1980, 1984, 1989; Morrow 1980; Reist 1989). This northern range limit is not definite and there is disagreement over the taxonomy, relationships, and distributions of char in this region. We did not collect the traditional meristic data to immediately address these issues and do not presently extend our analyses or discussion to include this area.

In the contiguous United States, Dolly Varden only occur in the most northwesterly drainages (Fig. 7A). We examined one fish (sample 11) from a Columbia River drainage in Washington that is a Dolly Varden. Since no other Dolly Varden were found in any Columbia River drainage, it is likely that this single record is in error or a recent headwater crossover. In addition, Morton (1970), Cavender (1978), and Goetz (1989) listed Dolly Varden from the Puget Sound and Olympic Peninsula regions of Washington (also T. Johnson, Department of Wildlife, 8594 Highway 101, Port Townsend, WA 98368, USA, pers. comm.). Although we examined no char from these two areas, they most probably contain Dolly Varden. This southern range of Dolly Varden appears to correspond to the southern extent of the Wisconsin (last Pleistocene) glaciation (Armstrong et al. 1965; McPhail 1967; McKee 1972), and the Olympic Peninsula was another distinct glacial refuge (Thorson 1980; McPhail and Lindsey 1986).

This southern distribution is consistent with Cavender (1978), except that he listed Dolly Varden from the McCloud River system in northern California. The identity of these McCloud River fish could not be verified, as they are so badly disintegrated that the U.S. National Museum of Natural History would not remove them from the jar for shipment or examination (D. Markle, Department of Fisheries and Wildlife, Oregon State University, Corvallis, OR 97331, USA, pers. comm.). Given our present distribution of Dolly Varden, these specimens are probably in error or at least difficult to explain. It seems unlikely that Dolly Varden naturally occurred that far south. The McCloud River char, whether they were Dolly Varden or bull trout, are now extinct (Hesseldenz 1985; Goetz 1989; M. Rode, California Department of Fish and Game, 3 N. Old Stage Rd., Mount Shasta, CA 96067, USA, unpubl. data).

The eastern range of Dolly Varden extends only to approximately 120°W, with the majority of samples being coastal and often anadromous (Fig. 7B). Dolly Varden occur in no east-flowing drainages except for our sample sites 73 and 74 which are in the extreme headwaters of the Liard River, British Columbia (≈130°W). These two sites are represented by a total of eight fish and it is thus more difficult to dimisss them as an error. They are, however, the only known Dolly Varden in a drainage flowing east off the North American Continental Divide. This information coupled with their extreme headwater location suggests that they may be a recent crossover.

Cavender (1978) also listed a Dolly Varden sample from a tributary of the Liard River. This sample consists of one fish and is located so much farther east (≈125°W) than any of our Dolly Varden collections in this region that it is difficult to explain zoogeographically. Although we do not have a large number of samples from this area, they are all bull trout. Thus, we assume that his record is in error.

Islands off both British Columbia and Alaska are occupied exclusively by Dolly Varden (Fig. 7C). In Alaska, Dolly Varden have a strong coastal distribution in spite of the apparent absence of bull trout in interior regions. The westernmost sample locality for Dolly Varden is St. Matthew Island, Alaska (sample 148), at approximately 172°E. Since Dolly Varden are present in Japan, this westernmost sample must be viewed only from our North American perspective.

Bull trout (S. confluentus)

Bull trout originally ranged south to approximately 41°N, but most of these southern populations are extinct and their present southern limit is about 42°N (Fig. 7A). The range of bull trout in the contiguous United States has become greatly restricted in recent times (Goetz 1989) and this species is now on the category 2 endangered list in the United States (Williams et al. 1989). Category 2 indicates a proposal to list the species as threatened or endangered but current evidence is inconclusive. Bull trout still exist as far south as the Oregon–California border

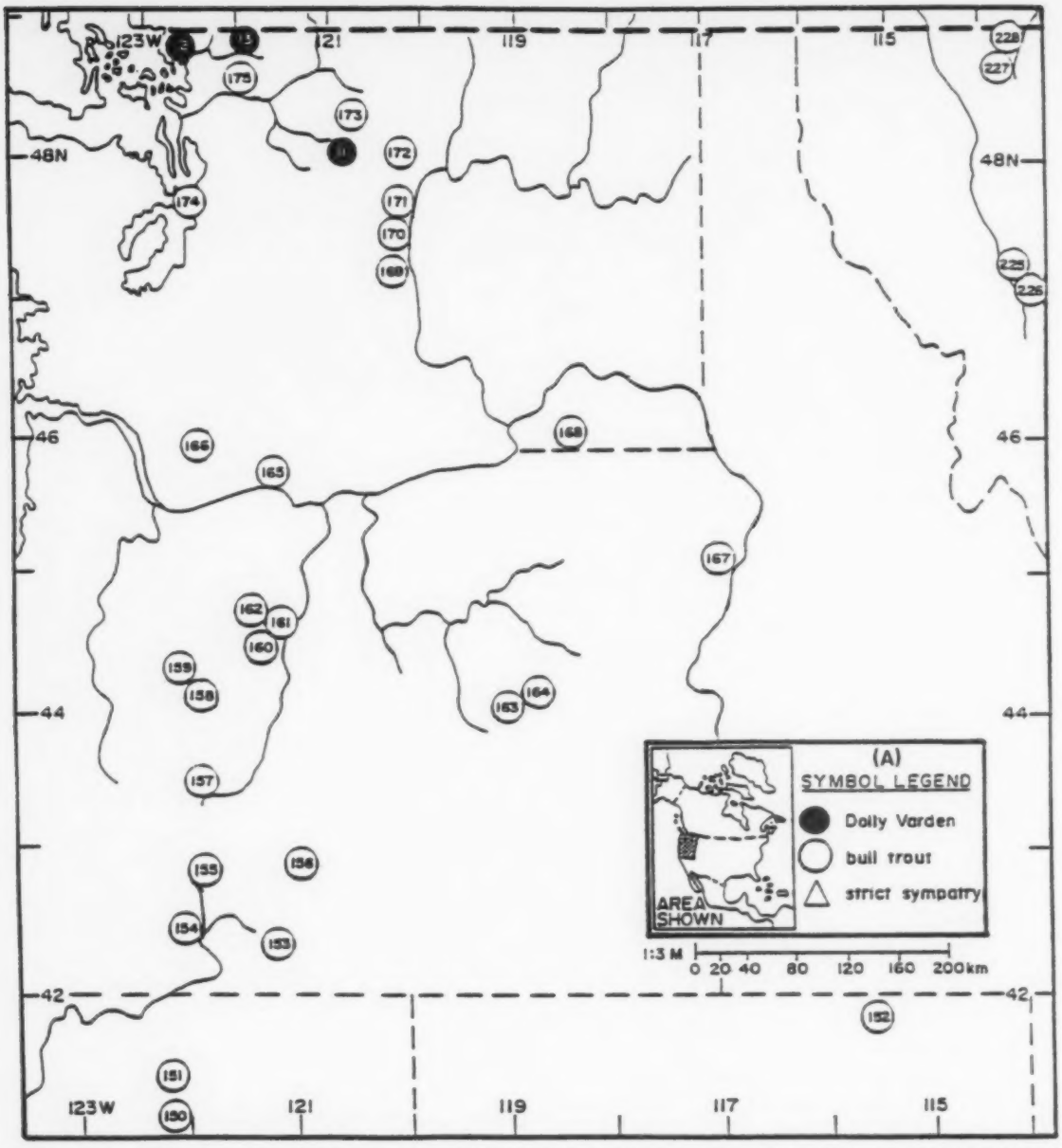


FIG. 7. Distributions of Dolly Varden and bull trout, as sampled in this study, in (A) the contiguous United States, (B) Canada and the Alaskan panhandle, and (C) Alaska. For sample site locations, see Appendix A.
(Fig. 7. continued next page)

but are no longer present in California and are also almost gone in Nevada. Local extinction does not yet appear to be a problem in Canada except in developed regions. Indeed, as one moves north through the ranges of bull trout and Dolly Varden, these char appear to increase in abundance and in the number of sites where they occur (also noted for Arctic char in Johnson 1980, 1989). This is probably due to the more pristine and suitable environments in northerly regions. In spite of their relative overall abundance, their gradual disappearance in the south indicates that they are environmentally sensitive and that care should be taken in their management (Goetz 1989).

The northern limit of bull trout is at about 60°N, but we have one sample (308) of five fish from approximately 61°N (Fig. 7B). This sample and another sample (307) are from the Yukon River drainage and suggest that bull trout could occur even farther north (also see McPhail 1961; Crossman and McAllister 1986; Lindsey and McPhail 1986). We have no further evidence, however, of bull trout in Alaska or in the Yukon Territory.

The most westerly bull trout samples are from northwestern British Columbia at approximately 133°W (Fig. 7B). Bull trout are largely confined to interior regions throughout their distri-

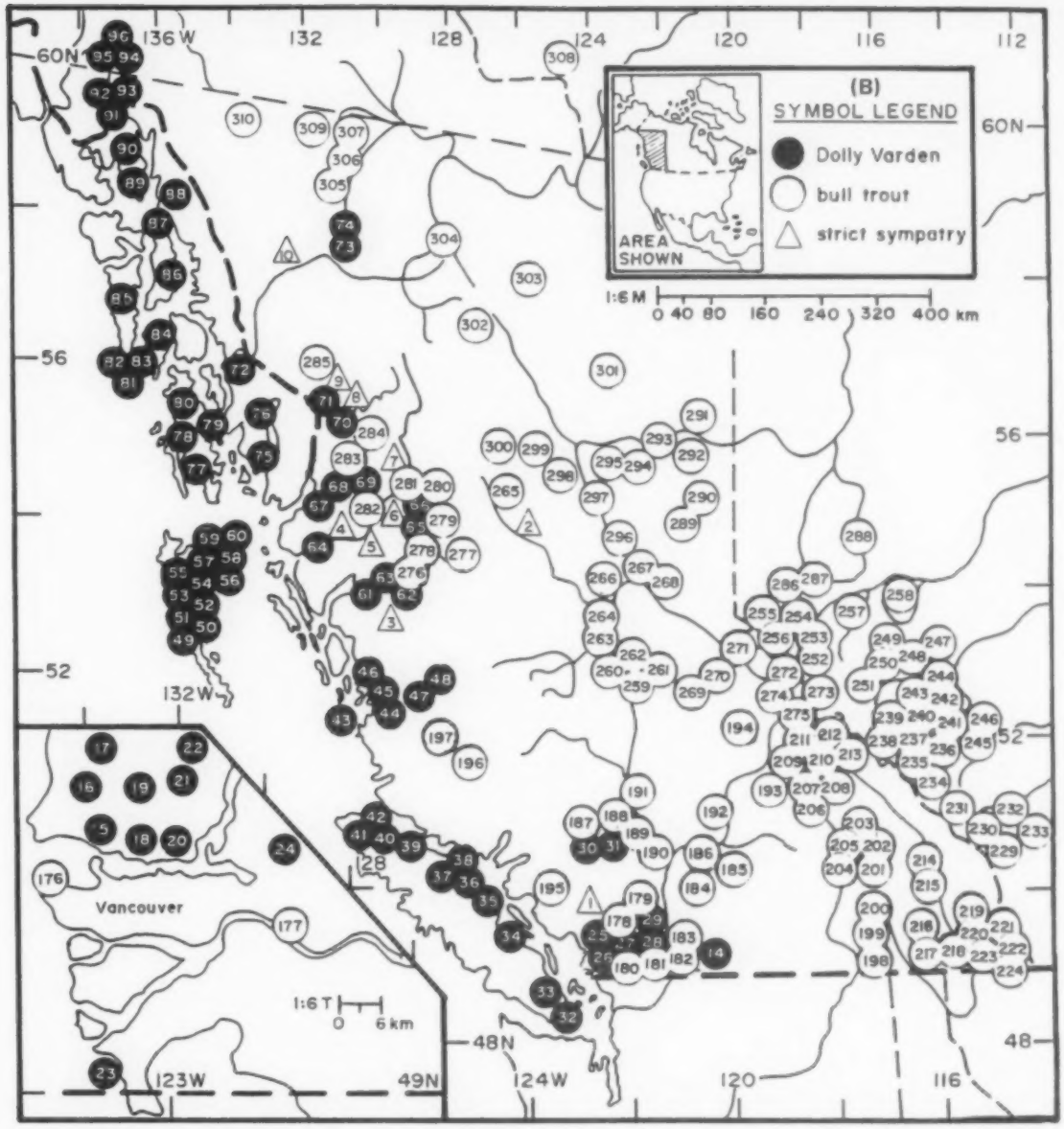


FIG. 7. *(Continued)*

bution. They only reach the actual Pacific coast in the Puget Sound area of Washington (Fig. 7A) and in the Fraser River drainage in British Columbia (Fig. 7B). We have a sample of bull trout from each of these regions that were collected in nearshore marine environments (samples 174 and 176), and Cavender (1978) also listed a nearshore marine sample from Puget Sound. Thus, it appears that bull trout can tolerate salinity. Some of our other coastal bull trout samples collected in freshwater also contained specimens displaying morphological and colour characteristics of sea-run salmonids. These bull trout may exhibit some form of migration but this is probably limited, since they have not been collected on any offshore islands or far out to sea. Dolly Varden certainly are the more anadromous of the two species (Cavender 1978).

Bull trout populations south of Puget Sound in Washington are not present in coastal regions (Fig. 7A; also see Goetz 1989). This is in spite of the absence of Dolly Varden from these southern areas. The only truly coastal and migratory bull trout are from regions where they occur with Dolly Varden. However, there are more northerly regions of co-occurrence between the two species near the actual Pacific coast, such as in the Skeena, Nass, and Stikine River drainages in British Columbia, where migratory bull trout are not present or were not detected (Fig. 7B). This difference in coastal distribution and migratory patterns between southern and northern populations of bull trout suggests some long-term differences between them or some influence on only the northern or southern group. The

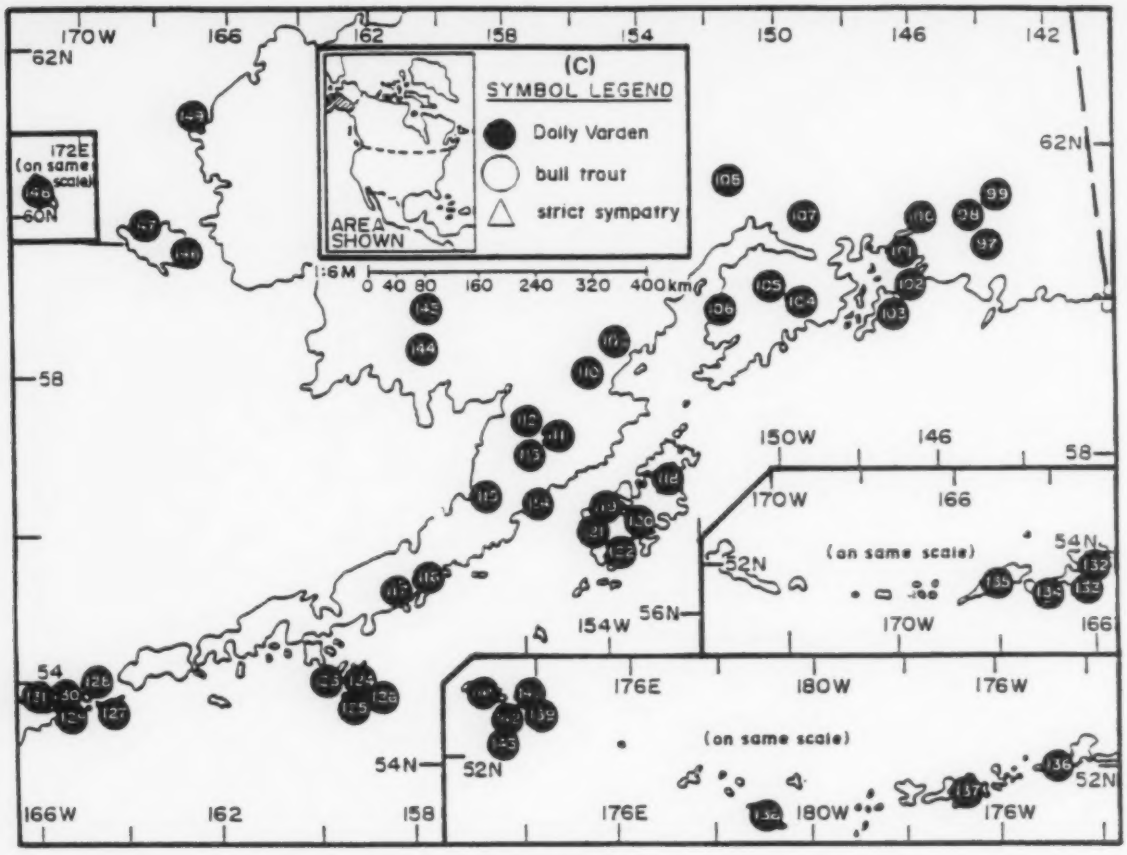


FIG. 7. *(Concluded)*

delineation between northern and southern bull trout seems to be at the southern extent of the Wisconsin (last Pleistocene) glaciation and is further indicative of changes built up over time. This delineation also corresponds to the southern range limit for Dolly Varden.

The eastern limit of bull trout is about 114°W, where they occur in western Alberta and western Montana (Fig. 7A and 7B). There is a much more easterly 1834 collection record from the Bear River (Rostlund 1951), a tributary of Great Salt Lake, but char are now extinct in this region (Goetz 1989). The Great Salt Lake area was once a Columbia River tributary, and this bull trout record could be real. However, the present and long-term absence of any char in this vicinity makes it appear dubious.

We found no bull trout specimens from the upper Missouri River system (contrary to Nelson 1977; Crossman and McAllister 1986). Bull trout occur in regions close to headwaters of the Missouri drainage, but we have no evidence of crossover or past occurrence. This distribution suggests that North American bull trout and Dolly Varden are western species that survived glaciation only within western refugia (Lindsey and McPhail 1986; McPhail and Lindsey 1986; G. R. Haas and J. D. McPhail, in prep.).

Acknowledgements

We particularly thank Dr. M. St. John and T. Suzuki for their help in collecting fish. Other field assistants were G. Birch, P. Haas, D. McLennan, M. Nevin-Haas, R. Sampson, and Dr. A. Tompkins. Char were collected and given to us by G. Birch, Dr. T. Down, Dr. C. Foote, M. Galesloot, Dr. G. Hartman, G. Hoyer, M. Peter, Dr. D. Webb, and the staff at the Capilano and Seymour River Hatcheries. G. Birch helped with the artificial crosses, and D. McLennan tended the laboratory fish in G.R.H.'s absence. The char drawings were done by K. Klitz. P. Haas assisted with the drafting of the maps, and A. Blachford helped with the digitized fish shape computer programs. The Regional Districts of Greater Vancouver and Dewdney-Alouette gave permission for collections in their restricted watersheds. Thanks also to the personnel at the various fish museums visited, especially Dr. D. Markle (OSU), W. Roberts (UA), L. Snyder (UW), and P. Sonoda and Dr. D. Catania (CAS). As well, we benefitted from discussions with T. Johnson, K. Kramer, Dr. D. Markle, B. T. D. Muttley, and F. P. Muttley. This research was supported largely by Natural Sciences and Engineering Research Council of Canada (NSERC) grant No. A6451 to J.D.M. but also by an Arctic Institute Grant-in-Aid to G.R.H. G.R.H. is greatly indebted to his wife, Molly, for helping make this lengthy study possible.

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Appendix A. Sample Site and Museum Storage Localities

Strict Sympatric Samples (Dolly Varden, *S. malma*, and bull trout, *S. confluentus*)

Fraser River drainages:
1. Pitt Lake (UBC)
2. Middle River (UBC, 2 samples)

Central coast/Fraser River drainage:
3. Tahtsa River (UBC)

Skeena River drainages:
4. Shames River (UBC, 2 samples)
5. Silburn Creek (UBC)
6. Seeley Lake (UBC)
7. Kispiox River (UBC)

Nass River drainages:
8. Meziadin Lake (UBC, 2 samples)
9. Surprise Creek (UBC)

Stikine River drainage:
10. Winter Creek (UBC)

Dolly Varden (*S. malma*)

Columbia River drainage:
11. Little Wenatchee River (UW)

Noosack/Skagit River drainages:
12. Nooksack River (CAS)
13. Canyon Creek (OSU)
14. Sumallo River (UBC, CAS)

Coastal mountain range drainages, southern British Columbia:
15. Capilano River (UBC)
16. Sisters Creek (UBC)
17. Phyllis Lake (UBC)
18. Lower Lynn Creek (UBC)
19. Lynn Creek headwaters (UBC)
20. Lower Seymour River (UBC)
21. Seymour Lake (UBC)
22. Upper Seymour River (UBC)

Fraser River drainages:
23. Roberts Bank (UBC)
24. Cedar Creek (UBC)
25. Loon Lake (UBC)
26. Katherine Lake (UBC)
27. McConnell (Cascade) Creek (UBC)
28. Dickson Creek (UBC)
29. Dickson Lake (UBC)
30. Birkenhead River (CAS)
31. Portage Creek (UBC)

Vancouver Island Drainages:
32. Sooke Lake (UBC)
33. Mesachie Lake (UBC)
34. Nile Creek (UBC)
35. Oyster River (UBC)
36. Quinsam Lake (UBC)
37. Greenstone Creek (UBC)
38. Kelsey Bay (UBC)
39. Kokish River (UBC)
40. Misty Lake (UBC)
41. Beaver Lake (UBC)
42. Keogh River (UBC)

Central coast drainages, British Columbia:
43. Kayne River (UBC)
44. Port John (UBC)
45. Link River (UBC)
46. Martin River (UBC)
47. Bella Coola River (UBC)
48. Shootlie Creek (UBC)

Queen Charlotte Islands drainages:
49. Mosquito Lake (UBC, 4 samples)
50. Skidegate Lake (UBC, 2 samples)
51. Slatechuck Creek (UBC)
52. Lawn Hill Creek (UBC)
53. Mamin River (UBC)

54. Unnamed Creek (UBC)
55. Unnamed Creek (UBC)
56. Tlell River (UBC)
57. Drizzle Lake (UA)
58. Bird Lake (UA)
59. Hiellen River (UBC)
60. Unnamed Creek (UBC)

Skeena River drainages:
61. Kildala River mouth (UBC)
62. Nanika River (UBC)
63. Morice Lake creeks (UBC)
64. Exstew River (UBC)
65. Miller Creek (UBC)
66. Mission Creek (UBC)

Nass River drainages:
67. Ishkheenickh River (UBC)
68. Nass River (UBC)
69. Tseax River (UBC)
70. McLeod Creek (UBC)
71. Bear (Clements) Lake (UBC)

Stikine River drainage:
72. Snake River (OSU)

Liard River drainages:
73. Dease Lake (UBC)
74. Five Mile Creek (UBC)

Alexander Archipelago drainages:
75. Manzanita Lake (CAS)
76. Point Sykes Cove (UW)
77. Kasook Inlet (UW)
78. Beaver Creek (CAS)
79. Thorne Bay (UW)
80. Union Bay (UW)
81. Port Armstrong (UBC, 2 samples)
82. Little Port Walter (CAS, 3 samples)
83. Port Lucy (UBC)
84. Forefinger Bay (UBC)
85. Indian Creek (CAS)
86. Hasselborg Lake (CAS)
87. Admirality Island (UBC)
88. Mendenhall Lake (UBC)
89. Excursion Inlet (UW)
90. Adams Inlet creek (UBC)

Alsek/Kelsall Rivers drainages:
91. Seltat Creek (UBC, 2 samples)
92. Clear Creek (UBC)
93. Kelsall Lake (UBC)
94. Takhanne River (UBC)
95. Klushu Lake (UBC)
96. Dezadeash Lake tributary (UBC)

Gulf of Alaska drainages:
97. Tobay Lake (CAS)
98. Long Lake (CAS)
99. Sinona Creek (CAS)
100. Valdez (CAS)
101. Olsen Bay (UBC)
102. Makaka Point creek (CAS)
103. Hamming Bay (CAS)

Kenai Peninsula drainages:
104. Ptarmigan Lake (OSU)
105. Upper Russian River (CAS)
106. Deep Creek (UBC)
107. Willow Creek (CAS, 2 samples)
108. Lake Judd (CAS)

Alaska Peninsula drainages:
109. Pedro Creek (OSU)
110. Lake Iliamna tributaries (OSU, 2 samples)
111. One Shot Creek (UBC)
112. Hidden Creek (OSU)
113. Hidden Creek (UBC)
114. Cold Bay (UW)
115. Ugashik River (OSU)
116. Chignik Bay (CAS)
117. Chignik River (CAS)

Kodiak/Afognak Islands drainages:
118. Raspberry Strait (UW)
119. Weir Creek (UW)
120. Upper Thumb River (UW)
121. Karluk Lake (CAS)
122. O'Malley River (UW)

Shumagin Islands drainages:
123. Nagai Island (UBC, 2 samples)
124. Simeonof Harbor Creek (UBC)
125. Simeonof Harbor (UBC, 3 samples)
126. Shumagin Island (UBC, 2 samples)

Fox Islands (Aleutian Islands) drainages:
127. Tigalda Island creek (UBC)
128. Akun Island creek (UBC)
129. Aleutian Island (CAS, 2 samples)
130. Chernofski Bay (CAS)
131. Dutch Harbor (UW)
132. English Bay (UW)
133. Unalaska (CAS)
134. Unalaska Island (UBC)
135. Umnak Island (UBC, 2 samples)

Adreanof/Rat Islands (Aleutian Islands) drainages:
136. Atka Island (CAS, UBC, UW)
137. Adak Island (UBC)
138. Amchitka Island creek (UBC, 2 samples)

Near Islands (Aleutian Islands) drainages:
139. Chloe Pond (UBC)
140. Nizki Island (UBC)
141. Attu Island (UBC, 3 samples)
142. Agattu Island Lake (UBC)
143. Agattu Island Bay (UBC, 2 samples)

Being Sea drainages, Alaska:
144. Lake Nerka (OSU)
145. Kisarilik Lake (UBC)
146. Kanikstalikmuit (UBC)
147. Dooksook River mouth (UBC)
148. St. Matthew Island (CAS, UBC)
149. Igiak Bay (UBC)

Bull Trout (*S. confluentus*)

California drainages (relict):
150. McCloud River (CAS, 4 samples)
151. Mount Shasta Hatchery (CAS)

Nevada drainage:
152. Jarbridge River (CAS)

Klamath River drainages, Oregon:
153. Boulder/Brownsworth/Demming, 3 samples / Leonard Creeks (OSU)
154. Cherry Creek (OSU, 2 samples)
155. Sun Creek (OSU, 3 samples)
156. Long Creek (OSU, 2 samples)

Lower Columbia River drainages, Oregon/Washington:
157. Trapper Creek (OSU)
158. Anderson Creek (OSU)
159. McKenzie River (OSU)
160. Metolius River (OSU)
161. Jefferson Creek (OSU)
162. Candle/Jack/Roaring Creeks (OSU)
163. Big Creek (OSU)
164. Malheur River (OSU)
165. Pine Creek (OSU)
166. White Salmon River (UW)

Middle Columbia River drainages, Oregon/Washington:
167. Lick Creek (OSU)
168. Touchet River (UW)
169. Rock Island Dam, Columbia River (CAS)
170. Wenatchee, Columbia River (CAS)
171. Entiat Wier, Columbia River (CAS)
172. Methow River (UW)
173. Stehekin River (OSU)

Coastal drainages, Washington:
174. Golden Gardens, King County (UW)
175. Baker River (UW)

Lower Fraser River drainages:
176. Spanish Banks (UBC)
177. Pitt River mouth (UBC)
178. Gold Creek (UBC)
179. Chehalis Lake (UBC)
180. Vedder River (UBC, 4 samples)
181. Foley Creek (UBC)
182. Foley Lake (UBC)
183. Coquihalla River (UBC, 7 samples)

Middle Fraser River drainages:
184. Nicola River (UBC)
185. Nicola Lake (UBC)
186. Coldwater River (UBC)
187. Birkenhead River (CAS)
188. Lake Creek (CAS)
189. Seton Lake (UBC)
190. Anderson Creek (UBC)
191. Fraser River (UBC)
192. Kamloops Lake (UBC)
193. Yard Creek (UBC)
194. Anderson Lake (UBC)

Coastal drainages, British Columbia:
195. Alice Lake (UBC)
196. Bluff Lake (UBC)
197. One Eye Lake (UBC)

Upper Columbia River drainages, British Columbia/ Montana:
198. Salmo River (UBC)
199. Kootenay Lake (UBC)
200. Powder Creek (UBC)
201. Lardeau River (UBC)
202. Duncan River (UBC, 2 samples)
203. Trout Lake (UBC)
204. St. Leon Creek (UBC, 4 samples)
205. Mackenzie Creek (UBC, 3 samples)
206. Greely Creek (UBC)
207. Illicillewaet River (UBC)
208. Woolsey (Silver) Creek (UBC)
209. South Pass Creek (UBC)
210. Flat Creek (UBC)
211. Columbia River (UBC)
212. Blaeberry River (UBC)
213. Kickinghorse River (UBC)
214. Columbia Lake (UBC)
215. Whitetail Lake (UBC)
216. St. Mary Lake (UBC)
217. Moyie Lake (UBC, 2 samples)
218. Gold Creek (UBC)
219. Lizard Creek (UBC)
220. Flathead River tributary (UBC)
221. Shepp Creek (UBC)
222. Sage Creek (UBC)
223. Howell Creek (UBC)
224. Flathead River (UBC)
225. Inez Lake (OSU)
226. Seeley Lake (OSU)

South Saskatchewan River drainages:
227. Irabel (UW)
228. Kennedy Creek (UW)
229. Lower Kananaskis Lake (UA)
230. Smith-Dorrien Creek (UA)
231. Barrier Reservoir (UA)
232. Prairie Creek (UA)
233. Sheep River (UA)

North Saskatchewan River drainages:
234. Upper North Saskatchewan River (UA, 2 samples)
235. North Saskatchewan River (UA)
236. Siffleur River (UA)
237. Abraham Lake (UA, 5 samples)
238. Cline River/Reservoir (UA, 3 samples)
239. North Saskatchewan River (UA, 4 samples)
240. Malma Creek (UA)
241. Whiterabbit Creek (UA, 3 samples)
242. Haven Creek (UA)
243. Tershishner Creek (UA, 2 samples)
244. North Saskatchewan River (UA)
245. Clearwater River (UA, 2 samples)
246. Seven Mile Creek (UA)
247. Elk Creek (UA)
248. Chungo Creek (UA)
249. Unnamed Creek (UA)
250. Brown Creek (UA)
251. Southesk River (UA)

Athabaska River drainages:
252. McLeod River (UA)
253. Deerlick/Eunice Creeks (UA)
254. Cold Creek (UA)
255. Mason Creek (UA)
256. Rock Creek (UA)
257. Gorge Creek (UA)

258. Chickadee Creek (UA)

Upper Fraser River drainages:
259. Quesnel River (UBC)
260. Quesnel River (UBC)
261. Swift River (UBC)
262. Cottonwood River (UBC, 3 samples)
263. Hixon Creek (UBC)
264. Stone Creek (UBC)
265. Takla Lake (UBC)
266. Wright Creek (UBC, 2 samples)
267. Aleza Lake (UBC)
268. Hansard Lake (UBC)
269. Willow River (UBC)
270. Bowron River (UBC)
271. King Creek (UBC)
272. Robson River (UBC)
273. Yellowhead Lake (UBC)
274. Swift Creek (UBC)
275. Albreda Camp Creek (UA, UBC)

Skeena River drainages:
276. Morice Lake (UBC, 4 samples)
277. Bulkley River (UBC)
278. Dennis Lake (UBC)
279. Kathlyn Creek (UBC)
280. Suskwa River (UBC)
281. Bulkley River (UBC)
282. Kitsumkalum Lake (UBC, 2 samples)

Nass River drainages:
283. Nass River (UBC)
284. Nass River (UBC)
285. Bowser Lake (UBC)

Peace River drainages:
286. Lower Pierre Greys Lake (UA)
297. Rocky Creek (UA)
288. Little Smoky River (UA)
289. Mast Creek (UBC)
290. Murray River (UBC)
291. Stoddart Creek, Charlie Lake (UBC)
292. Hood Lake (UBC)
293. Peace River (UBC)
294. Avalanche River (UBC)
295. Misinchinka Swamp (UBC)
296. Tacheeda Lakes (UBC, 2 samples)
297. Parsnip River (UBC)
298. Sylvester Creek (UBC, 2 samples)
299. Germanson Lake (UBC)
300. Diver Lake (UBC)
301. Chowade River (UBC)
302. Toodoggone Lake (UBC)
303. Chesterfield Lake (UBC)

Liard River drainages:
304. Manson Creek (UBC)
305. Bass Creek (UBC)
306. Simmons Lake (UBC)
307. Bus Lake (UBC)
308. Prairie Creek (UBC)

Yukon River drainages:
309. Swan Lake (UBC)
310. Gladys Lake (UBC, 2 samples)

Appendix B. Morphometrics and Meristics

Reduced Data (51 Morphometric and 10 Meristic Characters)

Body morphometry:
1. standard length; as in Hubbs and Lagler (1964)
2. body depth; as in Hubbs and Lagler (1964)
3. body width; width at point body depth is measured
4. peduncle length; as in Hubbs and Lagler (1964)
5. peduncle width; width at point peduncle depth is measured
6. snout length; as in Hubbs and Lagler (1964)
7. predorsal length; as in Hubbs and Lagler (1964)
8. dorsal fin to adipose fin length; insertion of dorsal fin to origin of adipose fin,
9. adipose fin to caudal fin length; insertion of adipose fin to base of most dorsal caudal fin ray
10. pectoral fin to pelvic length; insertion of pectoral fin to origin of pelvic fin
11. pelvic fin to anal fin length; insertion of pelvic fin to origin of anal fin
12. anterior gular plate length; distance across gular plate at its anterior point
13. lateral line to dorsal depth; lateral line to dorsal body surface at origin of dorsal fin
14. lateral line to ventral depth; lateral line to ventral body surface below origin of dorsal fin

Fin morphometry:
15. adipose fin base length; origin to insertion of adipose fin
16. adipose fin length; base at centre of adipose fin to its furthest tip
17. adipose fin depth; distance from ventral to dorsal surface of adipose fin at its centre
18. dorsal fin base; as in Hubbs and Lagler (1964)
19. posterior dorsal fin height; distance from insertion of dorsal fin to furthest tip of posterior dorsal fin ray
20. anal fin base; as in Hubbs and Lagler (1964)
21. body to caudal fin fork length; posterior part of hypural plate to anterior part of caudal fin fork
22. inner pectoral fin length; insertion of pectoral fin to posterior tip of innermost pectoral fin ray
23. pectoral fin base; origin to insertion of pectoral fin
24. pelvic fin base; origin to insertion of pelvic fin
25. pelvic axillary process length; anterior to posterior point of pelvic axillary process

Head morphometry:
26. preopercle length; posterior edge of orbit to posterior edge of preopercle
27. opercle length; posterior edge of preopercle to posterior edge of opercle
28. nostril size; longest distance across nostril from one skin edge to another
29. orbit depth; distance from ventral to dorsal edge of orbit
30. orbit skin flap; anterior edge of orbit to posterior edge of orbit skin flap at its centre
31. eye length; anterior edge of eyeball to posterior edge of eyeball
32. pupil length; anterior edge of eye pupil to posterior edge of eye pupil
33. orbit to dorsal depth; distance from centre of orbit to dorsal body surface directly above it
34. orbit to ventral depth; distance from centre of orbit to ventral body surface directly below it

35. orbit to maxillary depth; distance from centre of orbit to dorsal edge of maxillary directly below it
36. orbit to nostril length; anterior edge of orbit to posterior edge of nostril
37. snout width; inside edge of left nostril to inside edge of right nostril
38. head width; dorsal edge at centre of left orbit to dorsal edge at centre of right orbit
39. premaxillary length; anterior tip of premaxillary to anterior tip of maxillary
40. anterior mandible depth; distance from ventral to dorsal edges of mandible at its most anterior point
41. anterior mandible distance; ventral inside edge of left mandible to ventral inside edge of right mandible at their most anterior point

Truss morphometry (all landmark points extrapolated to edge of body, see Fig. 1):

42. landmark 1 (tip of snout) to 2 (posterior edge of lower jaw)
43. landmark 1 (tip of snout) to 3 (posterior edge of orbit)
44. landmark 2 (posterior edge of lower jaw) to 3 (posterior edge of orbit)
45. landmark 2 (posterior edge of lower jaw) to 4 (posterior edge of opercle)
46. landmark 3 (posterior edge of orbit) to 5 (origin of pectoral fin)
47. landmark 5 (origin of pectoral fin) to 7 (origin of dorsal fin)
48. landmark 6 (origin of pelvic fin) to 8 (origin of adipose fin)
49. landmark 8 (origin of adipose fin) to 11 (dorsal/posterior edge of hypural plate)
50. landmark 9 (origin of anal fin) to 10 (ventral/posterior edge of hypural plate)
51. landmark 10 (ventral/posterior edge of hypural plate) to 11 (dorsal/posterior edge of hypural plate)

Meristics:

1. dorsal fin rays; as in Hubbs and Lagler (1964)
2. anal fin rays; as in Hubbs and Lagler (1964)
3. caudal fin rays; count of all caudal fin rays
4. pectoral fin rays; as in Hubbs and Lagler (1964)
5. pelvic fin rays; as in Hubbs and Lagler (1964)
6. branchiostegal rays; total branchiostegal ray count (Hubbs and Lagler 1964). Counts were made separately on the right and left halves
7. mandibular pores; as in Hubbs and Lagler (1964). They were exposed by drying with a towel and then marked with a felt pen run across the mandible. The pore at the end of each mandible was not counted (Cavender 1978)
8. lateral line; as in Hubbs and Lagler (1964)
9. gill rakers; all rakers, including rudimentary ones, were counted on the removed first right gill raker arch. Counts were made separately on the upper and lower arches
10. pyloric caeca; counted by removing each one

Bénéfices à court terme des mouvements anadromes saisonniers pour une population d'omble de fontaine (*Salvelinus fontinalis*) du Nouveau Québec

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Doyon, J.-F., C. Hudon, R. Morin et F. G. Whoriskey Jr. 1991. Bénéfices à court terme des mouvements anadromes saisonniers pour une population d'omble de fontaine (*Salvelinus fontinalis*) du Nouveau Québec. Can. J. Fish. Aquat. Sci. 48: 2212–2222.

Cette étude caractérise les mouvements anadromes saisonniers d'une population d'omble de fontaine (*Salvelinus fontinalis*) et en compare les caractéristiques biologiques et énergétiques avec des ombles estivant en rivière. Les mouvements de dévalaison enregistrés à une barrière de comptage pendant 3 ans étaient intenses au printemps mais continuaient jusqu'en automne, étant corrélés avec les hausses rapides du niveau de la rivière. Le caractère passif de la dévalaison est souligné par la petite taille des ombles en dévalaison, leur faible coefficient de condition et leur concentration à l'embouchure de la rivière. Le patron de montaison variait annuellement, en relation avec les conditions de niveau. Lors de la montaison, les ombles anadromes avaient un coefficient de condition supérieur et un contenu en eau inférieur aux poissons résidant en eaux douces, suggérant que la migration permet une accumulation estivale plus rapide de réserves énergétiques. Cependant, le caractère fortuit des mouvements anadromes, ainsi que la similarité des caractéristiques biologiques (croissance, taille à maturité sexuelle, fécondité, diamètre des oeufs) entre les ombles anadromes et résidents en eau douce suggèrent que les deux groupes appartiennent à la même population, dont la composante effectuant une migration annuelle pourrait être déterminée aléatoirement.

This study characterizes the seasonal anadromous movements of a brook charr population and compares its biological and energetic characteristics with charr spending summer in freshwaters. Downstream movements monitored at a counting fence over 3 yr were most intense in spring but occurred until fall and were positively correlated with rapid increases of water level. The timing of movements varied from year to year. Smaller charr were most subject to being swept downstream compared with freshwater residents, and most trout were concentrated near the mouth of the river. These patterns suggest that the downstream movements of charr in this system are passive. Upstream migrants had a higher condition factor and a lower tissue water content than freshwater residents, indicating that downstream movements result in a faster accumulation of energetic reserves during summer. However, the fortuitous character of anadromous migrations as well as the absence of differences in the biological characteristics (growth, size at maturity, fecundity, egg size) suggests that anadromous and resident fish belong to a single population whose yearly migrant component could be randomly determined.

Reçu le 18 juin 1990
Accepté le 4 juin 1991
(JA615)

Received June 18, 1990
Accepted June 4, 1991

Les poissons sont qualifiés d'anadromes lorsqu'ils passent une partie de leur cycle vital en milieu marin et reviennent en eau douce pour se reproduire (McDowall 1987). Chez les salmonidés, le genre *Salvelinus* est celui qui démontre l'anadromie la moins prononcée, étant caractérisée par des séjours en mer de courte durée, particulièrement réduits chez les populations nordiques (2–4 mo, Power 1980). Le séjour en mer de l'omble de fontaine anadrome (*Salvelinus fontinalis*) se limite aux abords de leur rivière d'origine (Dutil et Power 1980), potentiellement relié au faible développement de la smoltification (McCormick *et al.* 1985). Ces éléments suggèrent que les mouvements de cette espèce sont plutôt de nature passive que déterminés (McCormick et Naiman 1984; Swanson 1986; Swanson et Kansas 1987).

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Si les mouvements de l'omble de fontaine anadrome sont passifs, nous devrions observer des mouvements de dévalaison et de montaison durant toute la saison estivale, sans patron particulier, ainsi que des corrélations significatives entre la dévalaison et l'augmentation de niveau de la rivière. Si les mouvements sont déterminés, nous prédisons des mouvements de dévalaison et de montaison très brefs et concentrés dans le temps, étant donné la courte durée de l'été à ces latitudes, permettant de maximiser la période du séjour en mer. De même, ces mouvements devraient être indépendants du niveau et des autres facteurs abiotiques.

Chez l'omble de fontaine, les avantages des migrations anadromes ont été reliés à la croissance accrue en mer (Whoriskey *et al.* 1981; Castonguay *et al.* 1982), permettant aux géniteurs d'atteindre une plus grande taille, une fécondité plus élevée (Montgomery *et al.* 1989) et une meilleure survie que les poissons résidant en eaux douces (Naiman *et al.* 1987). La migration anadrome pourrait avoir une importance accrue dans les régions nordiques, permettant le remplacement des réserves énergétiques nécessaires à la survie hivernale (Cunjak et Power 1986; Dutil 1986).

Cette étude compare les caractéristiques biologiques et énergétiques d'ombles de fontaine anadromes ou résidant en eau douce dans une rivière de la toundra sub-arctique. Nous examinons d'abord le synchronisme et la durée de la dévalaison, de la montaison, la durée du séjour en mer ainsi que l'influence de certains facteurs abiotiques (température de l'eau, niveau, pluie, etc.) sur le patron des mouvements. Nous comparons ensuite les caractéristiques biologiques (croissance, taille à maturité, fécondité etc.) et l'accumulation estivale d'énergie pour les ombles anadromes ou ayant résidé en eau douce. Ces informations nous permettent de déterminer si l'anadromie est un phénomène actif ou passif pour une population nordique d'omble de fontaine et son importance comme mécanisme de gains énergétiques.

Site d'étude

Rivière au Saumon est une petite rivière boréale située à la limite septentrionale (54°38'N, 79°35'W, fig. 1) de la baie James. La superficie du bassin de drainage est de 92 km^2. De l'embouchure au premier lac, le troçon principal mesure environ 8 km, a une largeur moyenne de 21,9 m et une profondeur moyenne de 30 cm. Le fond en est de sable, de gravier, de cailloux et de blocs. La période de dégel de la rivière va de mai à la fin octobre, tandis qu'elle s'étend de la mi-juin à décembre pour la baie James. La période de dégel printanier et les pluies automnales sont celles où le niveau est maximal dans la rivière. Rivière au Saumon se déverse dans la baie James via un lagon peu profond (profondeur maximale = 1 m). Le lagon subit périodiquement des apports d'eau salée provenant de la baie James (maximum 24 ppm), causant des fluctuations importantes de salinité au fond (6–21 ppm).

Ce cours d'eau est alimenté par plusieurs lacs, et coule en toundra sub-arctique, où le pergélisol descend rarement en deçà de 1 m de la surface du sol. A rivière au Saumon, la température de l'air durant la journée franchit les 10°C dès la fin mai, s'élève fréquemment à 20°C ou plus en juillet notamment, et parfois même jusqu'à 28°C (le 15 août 1987). Cependant la température de l'air chute rapidement durant la nuit, ce qui explique les températures peu élevées ainsi que les fortes variations de températures dans la rivière (fig. 2). Le bassin de rivière au Saumon est situé à 5 km de la jonction des baies de James et d'Hudson, ce qui l'expose à des vents $> 5\ m \cdot s^{-1}$ de 30 à 60 % des jours de juin à septembre (Doyon 1990).

Dans le bassin de rivière au Saumon, l'omble de fontaine cohabite avec une autre espèce anadrome, l'omble chevalier (*Salvelinus alpinus*). Les communautés lacustres se composent du touladi (*Salvelinus namaycush*), du grand corégone (*Coregonus clupeaformis*), du cisco (*Coregonus artedii*) et du meunier noir (*Catostomus commersoni*), alors que dans le lagon et la baie, on retrouve l'épinoche à neuf épines (*Pungitius pungitius*), l'épinoche à trois épines (*Gasterosteus aculeatus*), le chaboisseau à quatre cornes (*Myoxocephalus quadricornis*), le grand corégone, le cisco et la morue du Groenland (*Gadus ogac*).

Matériel et méthodes

Les conditions climatiques journalières ont été mesurées matin et soir (d'après Environnement Canada 1978). La température de la rivière au niveau du camp (1 m), du lagon (1 m, station 6) et de la baie (2,5 m, station 2) furent enregistrées en continu avec des thermographes (Ryan modèle J, données disponibles dans Walker *et al.* 1987 et Gregory *et al.* 1988, fig. 1). Le niveau de la rivière (centimètres) et la vitesse du courant (mètres par seconde) étaient mesurés quotidiennement, au moyen d'un mètre et d'un débitmètre (General Oceanics Inc. modèle 2030) respectivement. Les variations de niveau d'eau du lagon ont été mesurées avec un marégraphe (Service Hydrographique du Canada, modèle MSBF-0519), au cours de la saison de 1987.

Dans cette étude, on qualifie d'anadromes les poissons se déplaçant de l'eau douce à l'eau salée, peu importe si les mouvements sont actifs ou passifs. Les mouvements de dévalaison et de montaison des poissons anadromes ont été suivis au moyen d'une barrière de comptage, dont les barreaux étaient espacés de 1,4 cm en moyenne, munie de deux trappes orientées vers l'aval et vers l'amont (Kristofferson *et al.* 1986). La barrière fut installée à environ 80 m en amont de l'embouchure de la rivière (fig. 1), pour les saisons 1986 (5 juin au 24 septembre) et 1987 (27 mai au 22 octobre). En 1988, la barrière fut déplacée de 50 m vers l'amont, munie d'une seule trappe pour échantillonner la montaison (11 juillet au 11 octobre).

Les trappes furent vérifiées chaque jour à 09:00, à 14:00 et au coucher du soleil (19:00–22:00). Les poissons capturés à la trappe étaient mesurés (longueur à la fourche, mm), pesés ($\pm$ 20 g), étiquetés et relâchés. Les ombles les plus vigoureux ont été anesthésiés au MS 222 pour faciliter leur mesure. Les poissons de taille > 200 mm étaient étiquetés (1986: Carlin (Saunders 1968); 1987: Floy Anchor (Dell 1968)), tandis que les individus < 200 mm étaient marqués en coupant une nageoire. Un omble à tous les 10 passant à la barrière fut prélevé pour déterminer les caractéristiques biologiques (âge, sexe, maturité sexuelle, fécondité, diamètre des oeufs) (1986 à 1988) et énergétiques (contenu calorimétrique, contenu en eau) (1987) lors des mouvements saisonniers.

Durant l'été, les ombles ayant dévalé étaient échantillonnés dans le lagon, l'estuaire et sur la côte de la Baie James. Dans le lagon, des verveux (maille de 10 mm) et des filets maillants expérimentaux (1 $\times$ 50 m; six panneaux de 8,3 m de maille étirée de 25 à 102 mm) furent mouillés (fig. 1) pour 24 h hebdomadairement (1986) ou bi-mensuellement (1987) de fin mai à octobre. Selon leur état, les ombles capturés étaient soit échantillonnés, soit mesurés, étiquetés et relâchés, en notant les recaptures provenant de la rivière. A l'embouchure du lagon

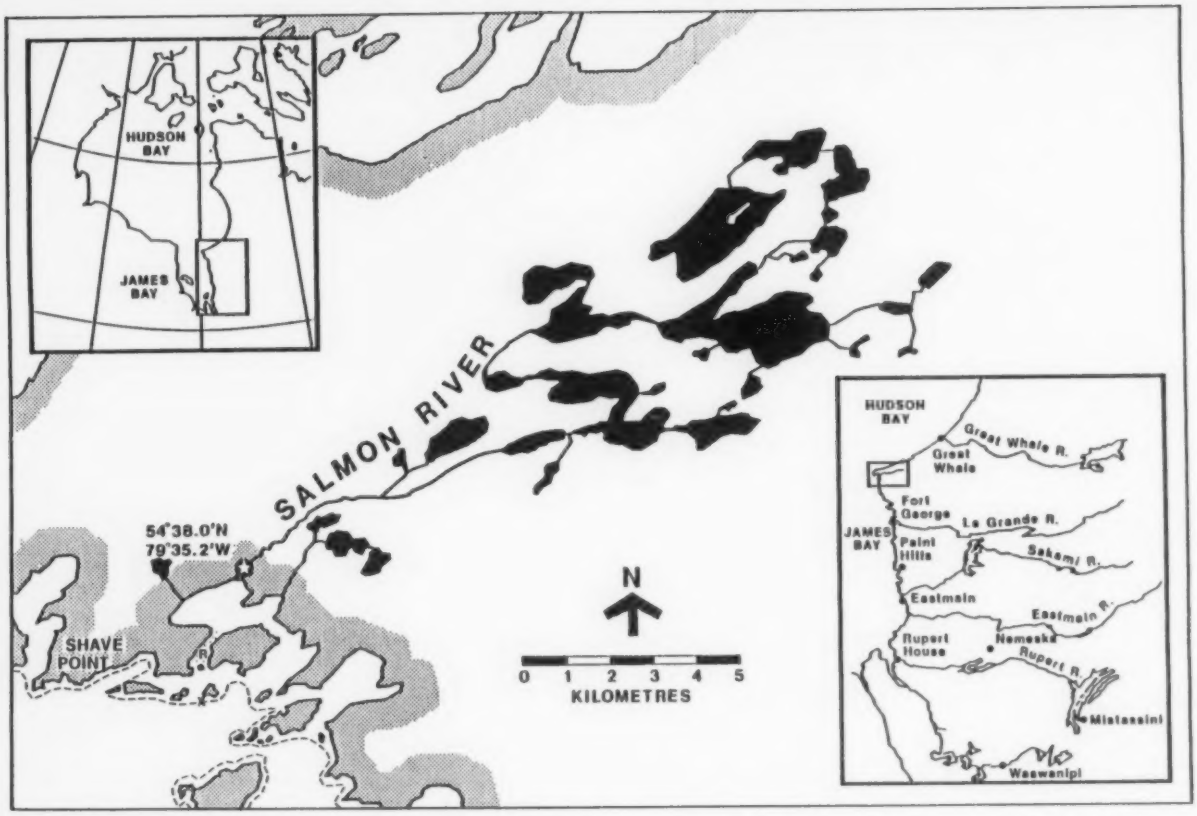

FIG. 1. Le bassin de la rivière au Saumon (Baie James, Nouveau Québec, encadrés). L'échantillonnage a eu lieu sur la côte de la Baie James, à l'embouchure du lagon, dans le lagon, dans la rivière et dans les lacs. L'étoile indique le site de la clôture de comptage, non loin du camp.

et sur la côte (fig. 1), des filets expérimentaux semblables (2 × 50 m) furent mouillés pour 24 h à 3 m (marée haute), bi-mensuellement (1986 à 1988), et toutes les captures étaient échantillonnées. A chaque échantillonnage dans le lagon et dans la baie, on mesurait la température et la salinité (Beckman modèle RS5-3) de la surface au fond par intervalle de 1 m.

En 1987, les ombles résidant en eau douce durant l'été furent échantillonnés en juin, à la fin juillet et à la mi-septembre, permettant la comparaison des caractéristiques biologiques et énergétiques avec les ombles effectuant des mouvements anadromes (durant la dévalaison, au cours et après le séjour estival en mer). Les ombles non marqués capturés en lac ou en rivière après le 6 juillet (date correspondant à la fin du pic de dévalaison) furent considérés comme résidents en eau douce. Ces derniers furent capturés au moyen de filets maillants expérimentaux dans les lacs et à la pêche à la ligne dans la rivière (fig. 1). Ils étaient soit échantillonnés, soit mesurés (millimètres), marqués (Floy Anchor) et relâchés immédiatement.

Caractéristiques biologiques et énergétiques

Les otolithes prélevés pour la détermination d'âge (Dutil et Power 1977) furent submergés dans le salycilate de méthyle pour trois lectures, subséquemment vérifiées par deux autres personnes. Les ovaires furent pesés (± 0.1 g) et préservés dans le fluide de Gilson pour 2 sem, après quoi les oeufs des deux gonades furent énumérés. En 1987, après avoir pesé les deux ovaires, le droit fut prélevé pour l'évaluation de la fécondité et la mesure du diamètre des oeufs, et le gauche fut utilisé pour l'analyse du contenu énergétique. Le diamètre des oeufs fut estimé par la moyenne de 30 oeufs mesurés pour chaque omble femelle échantillonné. Le coefficient de condition (K) fut calculé par la formule suivante :

$$(1) \quad K = \frac{\text{Poids total (g)} \times 10^4}{\text{Longueur à la fourche (mm)}^3}.$$

L'index gonado-somatique (IGS) et l'index hépato-somatique (IHS) représentent le rapport du poids des gonades ou du foie par rapport au poids total de l'individu, exprimé en pourcentage. On calcula aussi le coefficient de croissance instantané en poids (CCIP).

Les échantillons de tissus (foie, muscle, oeufs) provenant d'ombles > 300 mm furent prélevés en 1987 pour l'analyse du contenu énergétique. L'échantillon de muscle était prélevé du côté gauche de chaque omble, dans la région dorsale entre les nageoires anales et caudales. Les échantillons de tissus furent pesés (0,1 g), congelés à −7°C sur le terrain avant d'être expédiés au laboratoire, où ils furent entreposés à −15°C pour environ 6 mo. Les tissus furent lyophilisés à −60°C pour 4 jours, et pesés à nouveau pour en estimer le contenu en eau. Les tissus furent à nouveau entreposés à −15°C pour environ 2 mo, avant l'analyse calorimétrique. Le contenu calorimétrique des tissus secs fut déterminé avec une microbombe calo-

rimétrique Phillipson (Phillipson 1964). Chaque échantillon fut moulu au mortier et modelé en quatre pastilles (réplicats allant de 5 à 14 mg). Chaque pastille fut placée au four à 60°C pour 7–9 h pour éliminer l'humidité résiduelle et fut repesée (0,001 g) avant l'analyse. La bombe calorimétrique était recalibrée à l'aide d'un standard d'acide benzoique à toutes les 40 analyses.

Analyse statistique

L'analyse de coefficient de direction (path analysis, Legendre et Legendre 1984) a été employée pour tester l'hypothèse que la dévalaison survient passivement en relation avec les facteurs abiotiques. Ce genre de modèle permet d'inclure les relations indirectes existant entre les facteurs abiotiques, ce que ne peut faire la régression multiple. Cette analyse a été réalisée par le programme Piste du progiciel R (Legendre 1985) et permet de calculer les différents coefficients de direction et leur signification à partir d'une matrice de corrélations. Il est ainsi possible de décrire le réseau de relations entre les variables au moyen d'un modèle maximisant la portion de la variance expliquée.

Parmi les variables abiotiques, seulement celles directement liées au milieu aquatique (température de la rivière, du lagon, de la baie, précipitations, variation du niveau de la rivière et du lagon) furent retenues pour l'analyse effectuée sur les deux années regroupées. Ces critères furent sélectionnés pour obtenir un seul modèle utilisant les descripteurs les plus susceptibles d'expliquer les mouvements saisonniers des salmonidés (Castonguay *et al.* 1982; Dutil et Power 1980; Jonsson et Ruud-Hansen 1985; Jensen et al. 1986; Smith et Saunders 1958; White 1940). Pour permettre les comparaisons interannuelles des patrons de mouvements des ombles malgré les différences climatiques, la saison d'échantillonnage a été divisée en trois périodes de fluctuation des valeurs de niveau centrées et réduites (niveau — moyenne saisonnière/écart-type). Les trois périodes correspondent à la crue printanière (niveau centré-réduit > 0), à la sécheresse estivale (niveau centré-réduit < 0) et à la crue automnale (niveau centré-réduit > 0).

Pour la montaison, des corrélations non paramétriques de Spearman ont été calculées séparément pour chacune des années. Les corrélations de Spearman ont aussi été utilisées pour voir l'effet du niveau et de la température de l'eau sur la dévalaison automnale. Les analyses préliminaires permirent de diviser pour chaque année la saison d'échantillonnage en trois périodes distinctes correspondant aux fluctuations de niveau. La taille des ombles, le facteur de condition et l'IGS furent comparés au moyen d'analyses de variances à trois critères (année, station, période) au moyen du logiciel SAS (Proc GLM).

La taille atteinte pour les classes d'âge 1 à 6 fut comparée pour les ombles migrateurs et résidents au moyen d'analyses de variance à deux facteurs (année, sexe). La croissance des mâles et des femelles fut décrite par le modèle de von Bertalanffy. Les paramètres du modèle ont été ajustés par itération (logiciel SAS, PROC NLIN) en utilisant au départ les valeurs de croissance estimées par Dutil et Power (1980) pour les ombles de fontaine du Lac Guillaume-Delisle (Nouveau Québec).

La taille de 300 mm correspond à la limite inférieure de taille à maturité et a été utilisée pour délimiter les groupes lors des comparaisons de contenu énergétiques et de facteur de condition. Les comparaisons de contenu énergétiques entre ombles anadromes et résidents en eau douce furent faites au moyen d'analyses de variances à modèle hiérarchique (nested) pour tenir compte de la variabilité entre les réplicats au sein d'un même échantillon. L'analyse de variance non paramétrique de Kruskal–Wallis fut utilisée lorsque les conditions d'homoscédasticité n'étaient pas respectées.

Résultats

Facteurs physiques

A notre site d'étude, la variabilité à court terme en réponse aux conditions climatiques est nettement plus prononcée dans la rivière et le lagon que dans la baie. Par exemple, bien que la température de la rivière, du lagon et de la baie varient en parallèle (fig. 2), les écarts de température sont beaucoup plus prononcés dans la rivière, qui fluctue avec la température de l'air ($r = 0{,}86$; $P < 0{,}01$; $n = 184$). Le niveau de la rivière avait une réponse rapide aux précipitations (fig. 3): une chute de pluie de 22 mm en matinée (14 juin 1987) se manifestait le soir même par une augmentation de niveau de 8 cm dans la rivière ($r = 0{,}54$; $P < 0{,}01$; $n = 299$). De plus, on a observé des augmentations de 20 à 40 % du niveau en l'espace de quelques heures dans la rivière et le lagon lors de tempêtes. La salinité du lagon pouvait passer de 6 à 21 ppm à la suite des apports d'eau salée de la baie lors des marées de vives eaux.

Sur une base saisonnière, la température estivale de la baie est inférieure à celle de la rivière et du lagon, quoique ce patron soit inversé à l'automne, en raison du refroidissement plus lent des eaux de la baie (fig. 2).

Les variations résiduelles de niveau montrent que le dégel printanier et les pluies automnales amènent des valeurs de niveau supérieures à la moyenne estivale, tandis que les mois de juillet et août sont comparativements plus secs (fig. 3). Un examen des variations interannuelles (1986 à 1988) des conditions climatiques estivales indique une hausse de la température moyenne de l'air (7,56, 9,27 et 10,43°C respectivement) accompagnée d'une baisse de précipitations (296,3, 234,1 et 224,8 mm de pluie totale respectivement), résultant en 1988 en des températures nettement plus élevées et un très faible niveau d'eau dans la rivière. On peut aussi noter que la période de crue printanière s'étend sur une période comparable en 1986 et 1987 (pas échantillonnée en 1988), mais que le début de la crue automnale survient de plus en plus tardivement de 1986 à 1988 (fig. 3).

Patron de mouvements saisonniers des ombles anadromes

Dévalaison

En 1986, la barrière de comptage a été installée le 5 juin, soit environ 2 à 3 sem après la débacle, mais ce n'est que trois semaines plus tard (fin juin) qu'un pic de dévalaison fut observé, pour ensuite diminuer et cesser à la mi-juillet (fig. 3). En 1987, la barrière de comptage a été installée dès la débâcle (27 mai) et la dévalaison était manifestement amorcée, comme en témoigne le maximum de dévalaison dès la première semaine d'opération de la barrière, pour se terminer à la fin juin. On observe de plus quelques épisodes de dévalaison en été et en automne, particulièrement en 1987, où 32 % des mouvements de dévalaison furent observés en octobre (fig. 3).

Malgré l'installation hâtive de la barrière de comptage en 1987, le nombre total de poissons dévalant est resté similaire en 1986 et 1987 (tableau 1). Cependant, la taille moyenne des ombles dévalant était significativement plus petite en 1987, en raison de la représentation différente des individus < 150 et > 300 mm (tableau 1). Pour les deux années, on note que les

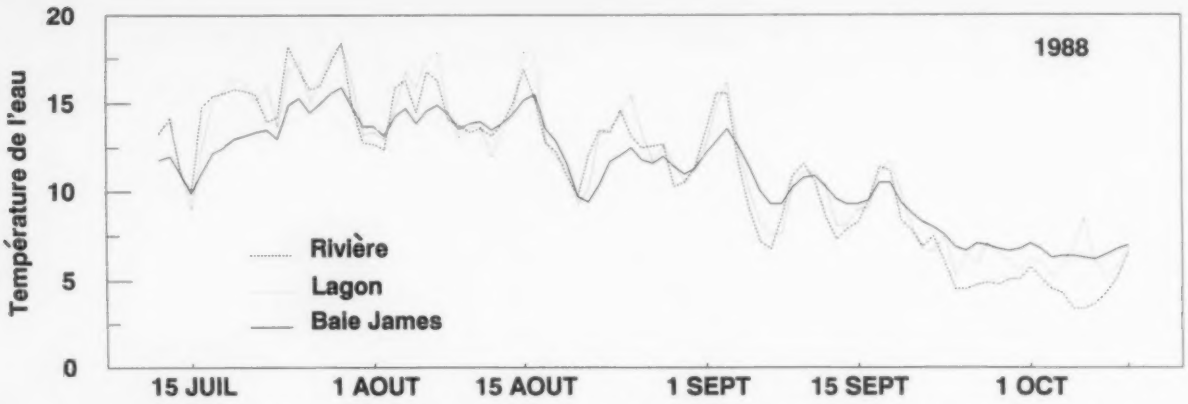

FIG. 2. Exemple des fluctuations journalières parallèles de la température à 1 m de profondeur sur la côte de la Baie James, dans le lagon et la rivière au Saumon durant la saison 1988.

ombles de plus petite taille dévalent durant la crue printanière, tandis que les plus gros poissons sont moins nombreux et se déplacent plus tard. La capture d'omble < 300 mm dans le lagon en juin 1986 avant le pic de dévalaison vient supporter cette observation (Doyon 1990). L'analyse de coefficient de direction révèle que la pluie, les variations de niveau et la température de la rivière expliquent près de 50 % de la variance des captures journalières en dévalaison pour les deux années re-groupées ($r = 0{,}69$, $P < 0{,}001$; $n = 120$ observations à la barrière). Ces descripteurs ont tous des effets positifs soient directs (niveau, température de l'eau, pluie), ou indirects (la pluie par l'intermédiaire du niveau). Etant donné la forte corrélation du niveau et de la vitesse du courant ($r = 0{,}98$; $P < 0{,}001$; $n = 270$), ce dernier facteur n'a pas été retenu dans l'analyse. Ainsi, après la débâcle, l'augmentation de la température et du niveau de la rivière lors de la fonte des neiges et des pluies printanières signalent le début de la dévalaison des ombles. Par ailleurs, les mouvements de dévalaison observés en automne 1987 sont corrélés positivement ($r_s = 0{,}34$; $P < 0{,}05$; $n = 74$) avec le niveau de la rivière et négativement avec la température de la rivière ($r_s = -0{,}42$; $P < 0{,}01$; $n = 74$). L'effet positif d'une augmentation rapide de débit sur la dévalaison se fait donc sentir peu importe la saison et la température de l'eau.

Montaison

Des ombles en montaison sont observés en petits nombres dès la mise en opération de la barrière de comptage, avec des pics plus marqués de juillet à octobre (fig. 3). La montaison est beaucoup plus prolongée et moins clairement définie que la dévalaison. L'activité de montaison est principalement distribuée entre les périodes 2 (bas niveau) et 3 (crue automnale). Cependant, le patron temporel de taille est affecté par la variation interannuelle dans la représentation des différents groupes de taille (tableau 1). En 1986, la prédominance des ombles > 300 mm résulte en une augmentation de la taille à la montaison durant l'été. En 1987 et en 1988, l'accroissement en nombre des ombles de 150–300 et < 150 mm respectivement altère considérablement ce patron. Les ombles de grande taille prédominent au milieu de l'été (période 2), tandis que les plus petits individus retournent en rivière à la fin de la saison (période 3, tableau 1). En 1988, le patron temporel des tailles des captures est altéré par l'abondance de petits individus (< 150 mm, tableau 1) dans la trappe, résultant en un pic d'activité de montaison (fig. 3) de poissons de faible taille (tableau 1) en période de sécheresse. De 1986 à 1988, on observe cependant une décroissance nette du nombre d'ombles > 300 mm en montaison, tout comme dans le cas de la dévalaison (tableau 1).

Le nombre total journalier d'ombles en montaison est significativement corrélé avec la pluie ($r_s = 0{,}32$; $P < 0{,}01$; $n = 367$) et les changements du niveau de la rivière ($r_s = 0{,}25$; $P < 0{,}01$; $n = 367$) pour les trois années d'études, tout comme la variation du niveau du lagon en 1987 (la seule année où elle fut mesurée) ($r_s = 0{,}33$; $P < 0{,}01$; $n = 149$). Les corrélations entre les mouvements de montaison et la température ne sont significatifs pour les trois années d'observations que pour la température de la rivière ($r_s = 0{,}13$; $0{,}01 < P < 0{,}05$; $n = 367$), révélant un retour en eau douce des ombles lors du refroidissement des eaux. Les relations avec la température du lagon et de la baie ne sont significatives qu'une année sur trois, et de sens inverse.

Séjour hors rivière

Pour les trois années d'échantillonnage, il existe une relation positive entre la taille moyenne des ombles capturés et la distance de l'embouchure de la rivière (fig. 4). On observe les plus fortes concentrations d'ombles dans le lagon et à son embouchure (tableau 2), ce qui correspond à des individus dont la taille moyenne est d'environ 250 et 300 mm respectivement (fig. 4). Les valeurs de CUE sont beaucoup moins élevées sur la côte de la Baie James (tableau 2), ce qui est relié à la faible abondance des ombles de taille > 350 mm (fig. 4). Notons qu'en 1987, année où la barrière de comptage a été installée juste après la débâcle, les CUE à l'embouchure du lagon étaient nettement plus faibles que les autres années.

Les données de marquage permettent de déterminer qu'il existe une corrélation négative entre la taille des ombles et la durée de leur séjour en mer ($r_s = -0{,}36$; $P < 0{,}05$; $n = 22$). Les petits individus effectuent une dévalaison précoce et une montaison tardive (tableau 1). De plus, le coefficient instantané de croissance en poids ($r_s = 0{,}62$; $P < 0{,}05$; $n = 14$) et le coefficient de condition ($r_s = 0{,}41$; $P < 0{,}05$; $n = 22$) des ombles anadromes étaient corrélés positivement avec la durée du séjour hors rivière. Notons que trois des poissons recapturés (11 %) perdirent du poids au cours de leur séjour hors rivière.

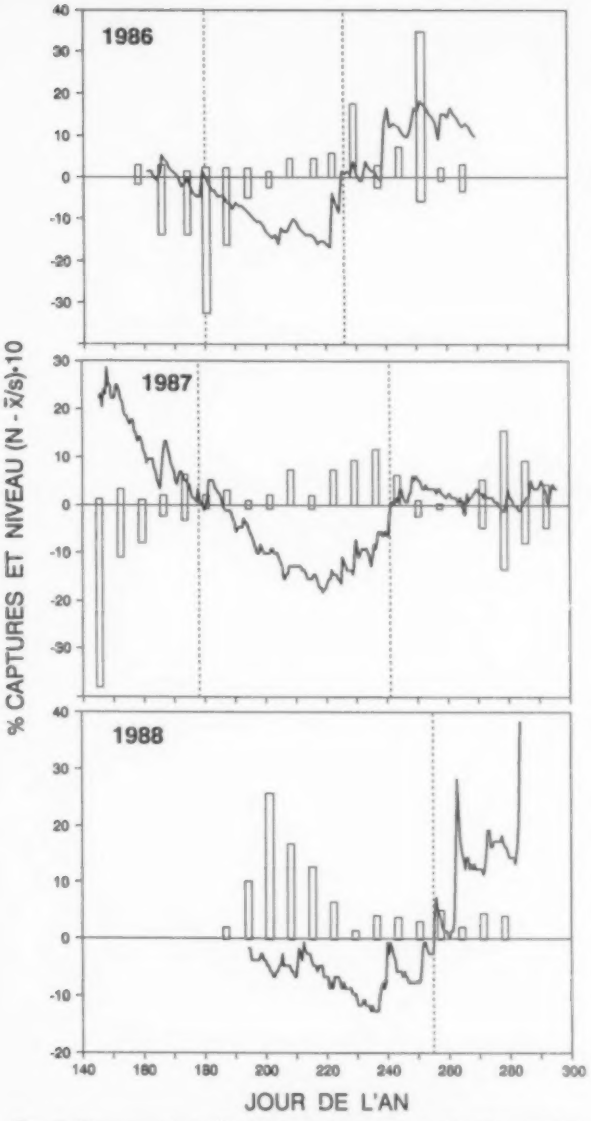

FIG. 3. Mouvements de dévalaison (valeurs négatives) et de montaison (valeurs positives) à la clôture de comptage en 1986, 1978 et 1988 (montaison seulement). La courbe superposée aux mouvements de chaque année indique les valeurs résiduelles de variations journalières du niveau de la rivière (niveau journalier − valeur moyenne pour toute la saison/écart-type).

Vingt pourcent des poissons étiquetés à la dévalaison en 1987 furent recapturés dans le lagon au cours de l'été tandis qu'en 1986 et 1987, trente pourcent des ombles ayant dévalé furent recapturés en montaison. Trois ombles parmi ceux étiquetés (3,5 %) ont été recapturés par des Cris, dont deux dans un lac situé à la pointe Louis XIV (5 km) et un individu dans la rivière Roggan (35 km).

Résidents en eau douce

Durant les trois saisons d'échantillonnage, un certain nombre d'ombles > 300 mm furent capturés en rivière en amont de la trappe en juillet et août, après que les mouvements intenses de dévalaison printanière eurent cessé. Les captures par unité d'effort (1 h de pêche à la ligne dans la rivière) étaient de 65,0 et de 31,7 poissons · d^{-1} en 1987 et 1988 respectivement. Pour les trois années d'échantillonnage, les résidents en rivière (moyenne = 391,8 mm; écart-type = 90,1; n = 696) et en lacs (345,6; 83,5; 271) avaient une taille significativement plus élevée que les ombles capturés aux autres stations (ANOVA, F = 84,10; $P < 0{,}001$). Les poissons capturés sur la côte (283,3; 89,5; 256) avaient une taille plus élevée que ceux capturés en montaison (247,8; 136,4; 585) et en dévalaison (240,5; 139,5; 215).

Comparaison des ombles anadromes et résidents en eau douce

Caractéristiques biologiques

Aucune différence significative de taille à l'âge (tableau 3) n'était discernable entre les ombles anadromes et les résidents en eau douce, ni entre les trois années d'étude, permettant de regrouper les observations des trois années.

L'équation de von Bertalanffy pour les mâles était de la forme :

$$LF = 541{,}025\,(1\text{-}e^{-0.307(t-0.206)})$$

et pour les femelles :

$$LF = 489{,}334\,(1-e^{-0.375(t-0.310)})$$

où LF représente la longueur à la fourche (millimetres) et t représente l'âge (années). Les paramètres de la courbe de croissance indiquaient une taille asymptotique de 541 mm pour les mâles et de 489 mm pour les femelles.

La taille à maturité n'était pas significativement différente entre les ombles anadromes et résidents. La taille à maturité des femelles allait de 313 à 522 mm tandis que les mâles allaient de 172 à 495 mm. A partir de l'âge de 5 ans (> 410 mm), 95 % des femelles (n = 212) et 85 % des mâles (n = 207) étaient matures. Ces mesures ont été faites sur des ombles provenant de tous les milieux, et indiquent que les ombles fraient à chaque année, plutôt qu'en alternance.

Aucune différence significative de fécondité ne fut observée entre les ombles anadromes et les résidents en eau douce. La relation entre la longueur et la fécondité des femelles de 3 à 7 ans est exprimée par la relation :

$$\text{Fécondité} = -6{,}646\ \text{longueur}^{2.358},\quad r^2 = 0{,}63;\ P < 0{,}05;\ n = 46.$$

Les femelles de 8 ans et plus ont été omises de l'équation de régression puisqu'un déclin de la fécondité semble être observé au delà de cet âge, indiquant possiblement la sénescence.

La taille des oeufs de poissons anadromes et résidents en eau douce fut mesurée à différents stades de développement, et doit par conséquent être corrigée en fonction de l'IGS pour éliminer l'effet de la période d'échantillonnage. Aucune différence significative du diamètre des oeufs n'était présente entre les poissons anadromes et résidents, la relation étant exprimée par l'équation :

$$\text{Diamètre des oeufs} = 0{,}196\ \text{IGS}^{0.381},\quad r^2 = 0{,}83;\ P < 0{,}05;\ n = 53.$$

L'IGS des femelles (> 300 mm) ne différait pas d'une année à l'autre mais était significativement plus élevé chez les ombles capturés en rivière (résidents et migrateurs groupés) que ceux des lacs et de la côte (tableau 4). Cette différence indique l'uti-

TABLEAU 1. Fréquence de taille, taille moyenne par période et taille moyenne annuelle des ombles de fontaine capturés en migration à la barrière de comptage au cours de chaque saison d'échantillonnage. Les périodes d'échantillonnage sont déterminées d'après les variations de niveau décrites à la fig. 3. Les valeurs annuelles de taille moyenne des poissons sont toutes significativement différentes ($P < 0{,}001$, valeurs transformées en $\log_{10}$). Le nombre total (n) de poissons capturés à la barrière de comptage pour chaque saison est indiqué.

Année	Période	Fréquence de taille (n)			Moyenne période	Moyenne année	Somme (n)
		< 150	150–300	> 300			
Montaison							
1986	1	0	5	7	319	385	131
	2	0	2	44	394		
	3	0	4	69	392		
1987	1	2	0	8	331	334	97
	2	1	2	43	388		
	3	4	19	18	283		
1988	2	228	57	17	131	146	357
	3	3	32	20	265		
Dévalaison							
1986	1	26	6	47	220	256	120
	2	1	7	15	317		
	3	0	3	15	381		
1987	1	29	2	17	157	191	95
	2	1	1	2	237		
	3	9	20	14	232		

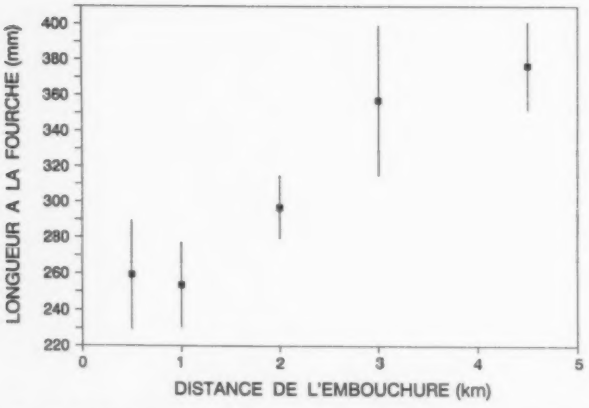

FIG. 4. Taille moyenne (et intervalle de confiance à 95 %) des ombles capturés à distance croissante de l'embouchure de la rivière au Saumon.

lisation de la rivière comme site de frai pour les ombles anadromes et résidents, et le déplacement de tous les individus matures vers la rivière à l'approche de la ponte. Les valeurs maximales de l'IGS sont atteintes lors de la sécheresse estivale, correspondant à la remontée précoce des géniteurs. L'examen des valeurs hebdomadaires de l'IGS pour les ombles femelles (> 300 mm) capturés dans la trappe et la rivière indique une augmentation linéaire (0,22 % par jour), entre le 27 juin et le 15 août, avec un maximum de 11,1. La ponte fut observée en rivière à compter du 28 août, et les premières femelles ayant terminé la ponte furent capturées le 7 septembre.

Contenu énergétique

Le coefficient de condition augmente de manière significative au cours de l'été (jour de l'année de juin à août) pour les ombles anadromes ($r = 0{,}38$; $P < 0{,}01$; $n = 228$) et les résidents en eau douce ($r = 0{,}24$; $P < 0{,}01$; $n = 138$) > 300 mm. Une comparaison plus détaillée du coefficient de condition des ombles < 300 mm à différentes stations au cours des trois périodes de l'été (tableau 5) indique que le coefficient de condition augmente à toutes les stations au cours de l'été mais que les ombles en montaison ont un coefficient de condition toujours supérieur à celui des autres stations. Les ombles en dévalaison et les résidents en rivière ont toujours les valeurs les plus basses.

TABLEAU 2. Capture par unité d'effort (CUE, nombre de poisson·filet^{-1}·jour^{-1}) des ombles de fontaine capturés au filet maillant à distance croissante (km) de l'embouchure de rivière au Saumon, de juillet à septembre. Le nombre de total de filet·jour^{-1} d'échantillonnage est indiqué entre parenthèses pour chaque valeur.

Station	1986	1987	1988	Moyenne
Lagon (0,5–1,0 km)	—	8,9 (18,4)	2,9 (8,7)	7,0
Embouchure du lagon (2,0 km)	44,0 (1)	3,6 (5,5)	25,0 (1,5)	8,2
Cote de la Baie James (3,0–4,5 km)	1,0 (5)	1,4 (9,2)	0,9 (9,2)	1,1

Le contenu en eau des tissus somatiques (muscle et foie) varie en sens inverse pour les ombles > 300 mm échantillonnés de juin à août : il diminue avec le temps pour les ombles anadromes ($r = -0{,}51$; $P < 0{,}01$; $n = 120$) mais il augmente pour les ombles résidents en eau douce ($r = 0{,}23$; $P < 0{,}01$; $n = 213$) > 300 mm.

Le contenu en eau des oeufs des ombles anadromes en montaison avant le frai était moins élevé que celui des ombles résidents en rivière durant la même période ($F = 9{,}11$; $P < 0{,}05$; $n = 9$ ombles anadromes et 16 résidents) (tableau 6). Cependant, le contenu énergétique des muscles, du foie et des oeufs

TABLEAU 3. Comparaison de la taille moyenne (écart type; *n*) atteinte à chaque âge pour les ombles anadromes et résidents en eau douce. La valeur critique du Test de t est indiquée. Aucune des différences de taille à l'âge entre poissons anadromes et résidents n'est significative ($P > 0,05$)

Age	Anadromes	Residents	*t*
1	127,83	110,0	0,445
	(37,09; 6)	(0;1)	
2	236,08	233,03	0,469
	(35,92; 40)	(25,00; 63)	
3	302,98	317,45	−1,801
	(41,00; 50)	(38,56; 49)	
4	359,65	373,67	−1,624
	(41,36; 32)	(36,67; 64)	
5	410,55	418,54	−0,840
	(28,78; 11)	(30,02; 59)	
6	487,00	437,56	1,840
	(0;1)	(26,15; 18)	
7	463,5	462,0	0,050
	(40,31; 2)	(27,19; 9)	

des ombles > 300 mm ne différaient pas entre les ombles anadromes et résidents (tableau 6), et d'importantes variations étaient enregistrées entre les réplicats d'un même échantillon. Le frai exerce une demande importante sur les réserves énergétiques à court terme des poissons d'eau douce (tableau 7). On observe chez ces derniers une diminution du facteur de condition (13 %), de l'IHS (21 %) et du contenu calorifique du muscle ainsi qu'une augmentation du contenu en eau du foie et du muscle (3 %). Le petit nombre d'ombles anadromes recapturés après le frai ($n = 2$) empêche les comparaisons pour ce groupe.

Discussion

Le patron saisonnier de dévalaison printanière et de montaison automnale observé à rivière au Saumon est similaire à celui précédemment rapporté pour d'autres rivières se jetant en milieu côtier (Murray 1968; Lear et Day 1977; Dutil et Power 1980; Steele 1986; Montgomery *et al.* 1989). De plus, nos résultats suggèrent que le schéma général des mouvements saisonniers (fig. 5) a des répercussions importantes sur les caractéristiques biologiques et énergétiques de la population d'ombles de fontaine de rivière au Saumon.

Les résultats de notre étude suggèrent que la dévalaison de l'omble de fontaine à rivière au Saumon est passive plutôt que déterminée. A la débâcle de la rivière (mi-mai) lors des plus hauts débits de la saison, on assiste à la dévalaison maximale des ombles, en particulier des individus de taille < 300 mm, dont la fréquence diminue à la fin juin. Les ombles en dévalaison sont en moins bonne condition que les ombles ne dévalant pas la rivière à cette époque, suggérant que seuls les poissons les plus faibles se laissent entraîner vers l'aval par l'augmentation de niveau. On assiste aussi à des épisodes de dévalaison d'ombles de faible taille lors des crues automnales, tout comme l'a documenté l'étude de Weir (1979) dans la rivière Wapiscau (Ontario, Bassin de la Baie d'Hudson). Qu'ils se produisent au printemps ou à l'automne, les épisodes de dévalaisons correspondent souvent à de faibles températures ambiantes, qui réduisent la capacité natatoire (Rimmer *et al.* 1985) et pourraient rendre les petits individus particulièrement sensibles à une augmentation de niveau se produisant simultanément.

Le caractère passif et la vulnérabilité des ombles de petite taille à la dévalaison sont aussi supportés par plusieurs observations suggérant que la barrière a exercé un effet détracteur sur les mouvements des ombles de fontaine, particulièrement en 1987 où la barrière fut installée dès la débâcle et maintenue en place pour la plus longue durée (148 jours). Tout d'abord, beaucoup moins d'ombles ont dévalé la rivière en 1987 (0,851 ombles · d^{-1} d'opération de la barrière) qu'en 1986 (1,108 ombles · d^{-1}). Cette différence se fait surtout sentir au niveau des individus > 300 mm (tableau 1), dont le nombre total passe de 77 à 33. L'effet négatif de la barrière sur la dévalaison des gros individus en 1987 se traduit par les plus faibles captures enregistrées sur la côte (tableau 2), les plus fortes captures d'ombles en rivière (2,7 ombles·h^{-1} à la pêche à la ligne), et la taille significativement plus élevée des poissons résidents en rivière.

Les poissons ayant dévalé la rivière séjournent en majeure partie dans le lagon et à son embouchure, où les ombles < 300 mm peuvent échapper à l'influence marine plus marquée de la Baie James jusqu'où se dispersent les ombles > 350 mm (fig. 4). Les ombles anadromes pourraient ainsi

TABLEAU 4. IGS moyen (écart-type; *n*) observé chez les ombles femelle (> 300 mm) capturés à chaque station pour l'ensemble des trois saisons d'échantillonnage divisées en trois périodes d'après les fluctuations de niveau (fig. 3). Les lettres indiquent l'appartenance à des groupes distincts d'après le test SNK sur les moyennes.

	Période				
	1	2	3	Global	Groupe
Montaison	0,79	8,55	5,64	7,52	A
	(0; 1)	(5,84; 12)	(6,70; 3)	(16)	
Rivière	0,77	8,01	2,75	6,62	AB
	(0; 1)	(5,18; 49)	(4,61; 16)	(66)	
Dévalaison	—	1,28	13,82	5,46	AB
		(1,03; 2)	(0; 1)	(3)	
Lac	1,00	4,26	2,04	2,81	CB
	(0,68; 30)	(4,57; 45)	(3,52; 14)	(89)	
Côte	0,65	1,10	0,37	0,89	C
	(0,32; 6)	(1,85; 21)	(0,06; 6)	(33)	
Global	0,93	5,52	2,64		
	(83)	(129)	(40)	(207)	
Groupe	A	B	C		

TABLEAU 5. Facteur de condition moyen (écart-type; *n*) des ombles < 300 mm capturés à chaque station au cours des trois saisons d'échantillonnage divisées en trois périodes d'après les fluctuations résiduelles de niveau décrites à la fig. 3. Les lettres indiquent l'appartenance à des groupes distincts d'après le test SNK sur les moyennes.

	Période				
	1	2	3	Global	Groupe
Montaison	1,01 (0,23; 3)	0,97 (0,13; 54)	1,22 (0,23; 22)	1,04 (79)	A
Lacs	0,98 (0,08; 8)	1,05 (0,20; 37)	0,99 (0,17; 39)	1,02 (84)	AB
Côte	0,83 (0,06; 11)	1,03 (0,08; 45)	1,01 (0,09; 30)	0,99 (86)	AB
Rivière	—	0,97 (0,14; 12)	0,95 (0,12; 28)	0,96 (40)	B
Dévalaison	0,83 (0,24; 42)	0,83 (0,27; 6)	1,08 (0,16; 9)	0,87 (57)	C
Global	0,86 (64)	1,00 (154)	1,03 (128)		
Groupe	A	B	B		

TABLEAU 6. Comparaison des caractéristiques énergétiques (contenu en eau, calorimétrie (cal·g poids sec^{-1}), IHS) avant le frai, chez les ombles matures (> 300 mm) ayant séjourné en mer (capturés en montaison) ou chez les résidents en eau douce. Le nombre de poissons analysés (*n*) et la présence de différence (ns = différence non significative entre les moyennes; *différence significative (Kruskal–Wallis, $P < 0,05$)) sont indiqués.

	Montaison	Résident
% eau dans les oeufs	62,39 (n= 9)	64,44 (n = 16)*
Calorimétrie		
Oeufs	5,761 (n = 9)	5,88 (n= 16) ns
Muscle	5,091 (n = 14)	4,870 (n = 13) ns
Foie	5,028 (n = 14)	5,081 (n = 13) ns
IHS	1,460 (n = 18)	1,661 (n = 22) ns

TABLEAU 7. Comparaison des caractéristiques énergétiques (coefficient de condition, IHS, contenu en eau et calorimétrie (cal·g poids sec^{-1})) des ombles (> 300 mm) résidents en eau douce capturés avant et après le frai en 1987. Le nombre de poissons sur lesquels les mesures ont été faites (*n*) ainsi que la présence de différences significatives entre les moyennes (ns = différence non significative; *différence significative dans le temps (ANOVA de Kruskal–Wallis, $P < 0,05$) ont été indiqués.

	Pré frai (août)	Post frai (mi-septembre)
Coeff. de condition	1,111 (n = 47)	0,966 (n = 18)*
IHS	1,652 (n = 47)	1,289 (n = 18)*
Contenu en eau (%)		
foie	79,02 (n = 47)	82,81 (n = 18)*
muscle	77,23 (n = 43)	80,23 (n = 19)*
Calorimétrie		
foie	5,081 (n = 12)	4,930 (n = 13)*
muscle	4,870 (n = 12)	4,983 (n = 14) ns

ajuster en fonction de leur taille le degré d'adaptation osmorégulatrice nécessaire lors de leur séjour hors rivière. Il faut noter que la plupart des individus de grande taille capturés en montaison n'arboraient pas la couleur argentée caractéristique des poissons migrateurs anadromes. Les bénéfices du séjour estuarien des ombles anadromes se manifestent principalement chez les ombles < 300 mm, par un accroissement estival plus rapide du coefficient de condition et du coefficient instantané de croissance en poids que chez les ombles résidents en eau douce. De plus, le contenu en eau des tissus somatiques des ombles anadromes décroît pendant l'été tandis qu'il s'accroît légèrement chez les résidents en eau douce. La faible teneur en eau des tissus de salmonidés ayant séjourné hors rivière a été corrélée avec leur contenu élevé en lipides (Cunjak et Power 1986; Cunjak 1988).

Le faible pourcentage de recapture en montaison (20–30 %) est explicable par plusieurs facteurs. Une partie des ombles dévalant la rivière au printemps le font avant l'installation de la trappe, et ne sont pas étiquetés. De plus, la trappe a une efficacité limitée pour la capture d'ombles de petite taille (< 150 mm). Certains ombles semblent aussi séjourner sous la glace dans les estuaires durant l'hiver sans effectuer de montaison à l'automne (F. G. Whoriskey, données non publiées). Enfin, une partie des ombles est échangée avec les rivières avoisinantes. Le taux de recapture dans les rivières avoisinantes (3,5 %) pourrait sous estimer le taux d'échange réel entre les rivières, en raison de l'effort de pêche très localisé des autochtones et des difficultés de récupération des étiquettes sur ce grand territoire. Le gradient de taille très marqué à distance croissante de l'embouchure (fig. 4) suggère cependant que seuls les ombles > 350 mm pourraient être susceptible de tels échanges. En conditions de sécheresse, où les faibles niveaux retardent la montaison (comme en 1988), une telle dispersion pourrait permettre aux ombles de remonter une rivière dont les conditions physiques (niveau, température) seraient plus favorables que celles de leur rivière d'origine (Weir 1979). L'altération du patron saisonnier de taille observé en 1988, ainsi que les différences annuelles des corrélations entre les variables physiques et les captures en montaison indiquent l'importance des conditions climatiques pour le comportement migratoire de l'omble de fontaine. Cette influence est particulièrement prononcée pour les rivières de petite taille telle que rivière au Saumon.

Au cours de la période de sécheresse estivale (période 2: juillet à août), on observe la montaison d'individus matures (taille moyenne de 394 et 388 mm en 1986 et 1987 respectivement) qui reviennent pour le frai. Ces ombles portent des

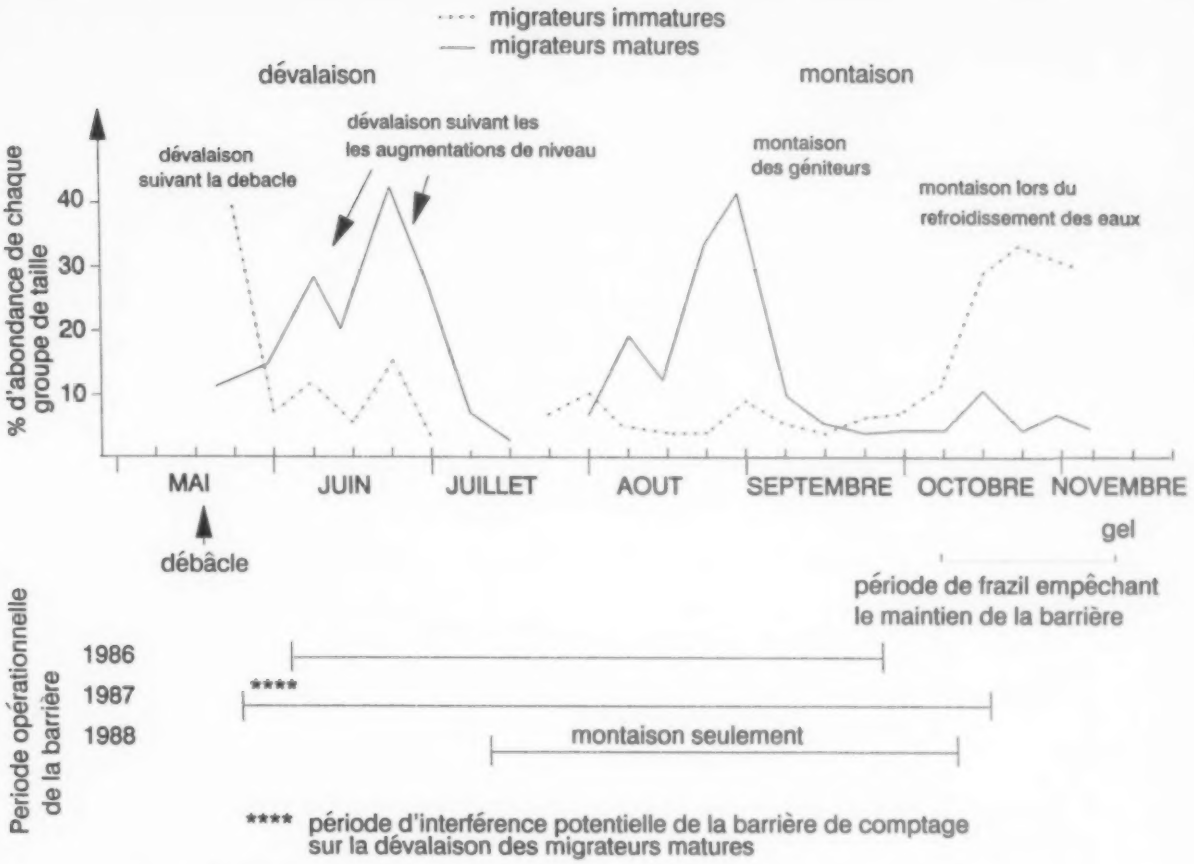

FIG. 5. Schéma général des mouvements saisonniers de l'ombles de fontaine anadromes de rivière au Saumon.

oeufs dont le contenu en eau est inférieur à ceux des résidents en eau douce (tableau 6), mais dont les caractéristiques énergétiques somatiques ne diffèrent pas de facon marquée de celles des poissons ayant passé l'été en eau douce. Exempts de la contrainte du frai, les ombles immatures sont ceux pour lesquels les bénéfices d'un séjour en mer sont les plus marqués. Contrairement aux poissons matures, ils remontent la rivière lors de la crue automnale (de septembre jusqu'au gel), permettant de maximiser la durée de leur séjour hors rivière (Castonguay *et al*. 1982; Steele 1986). Chez ces ombles, le coefficient instantané de croissance en poids et le coefficient de condition étaient corrélés positivement avec la durée du séjour hors rivière. La montaison des ombles ne se reproduisant pas pourrait être déterminée par le refroidissement des eaux, puisque les ombles ont une faible capacité d'osmorégulation à basse température (Saunders *et al*. 1975; Wandsvik et Jobling 1982).

La croissance de l'omble de fontaine à rivière au Saumon est plus rapide que celle rapportée pour d'autres rivières nordiques (Dutil et Power 1980). De plus, la ponte a lieu annuellement plutôt qu'aux années alternées, ce qui représente un élément de productivité supérieure pour rivière au Saumon. Plusieurs éléments peuvent être invoqués pour expliquer ces différences. En raison de sa localisation sur des dépots marins alcalins, la productivité limnicole du bassin de rivière au Saumon pourrait excéder celle de plus grandes rivières avoisinantes dont le bassin versant s'étend jusqu'au plateau intérieur (Legendre et Beauvais 1978). La capacité d'effectuer un séjour périodique en mer à différents moments de la vie d'un omble pourrait supplémenter la croissance individuelle, en comparaison avec des ombles nordiques résidant constamment en eau douce.

Le caractère fortuit des mouvements anadromes, ainsi que l'absence de différence de caractéristiques biologiques (crossance, taille à maturité sexuelle, fécondité, diamètre des oeufs) entre les ombles anadromes et ceux résidents en eau douce, permettent de supposer que les deux groupes de poissons appartiennent à une seule et même population. On peut s'interroger quant à la valeur adaptative d'un séjour en mer pour les poissons matures en l'absence de bénéfice énergétique net. Une possibilité serait de rendre la population reproductrice moins susceptible à une mortalité massive. Le groupe d'ombles effectuant une migration anadrome pour une saison donnée pourrait ainsi représenter un sous-ensemble « vulnérable à la dévalaison » de la population, déterminé plus ou moins aléatoirement par la combinaison de facteurs tels que le coefficient de condition individuel, la capacité natatoire selon la température ambiante et/ou la localisation physique des poissons au moment où surviennent les crues. Une étude plus approfondie des facteurs déterminant la fréquence des séjours hors rivière au cours

de la vie des individus pourrait amener de nouvelles perspective sur le développement de l'anadromie chez l'omble de fontaine.

Remerciements

Le support technique et les conseils de S. Leach et D. Hope furent très appréciés, de même que l'aide de J. Fife pour l'opération de la microbombe calorimétrique. B. Gagnon, C. Lameboy, B. Morin, S. C. Riley, A.-M. Savaria et la famille Snowboy de Chisasibi ont aidé à la collecte des données sur le terrain. Cette étude fut soutenue financièrement par la Station de Biologie Arctique du ministère des Pêches et des Océans, par les fonds F.C.A.R. et le centre des études nordique et de la recherche de l'université McGill. Les auteurs remercient M. Curtis et E. M. P. Chadwick pour leurs commentaires sur les versions antérieures du manuscrit.

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Morphological Basis of the Pectoral Fin Flutter of Embryonic Atlantic Salmon (*Salmo salar*)

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Peterson, R. H., J. Power, and D. J. Martin-Robichaud. 1991. Morphological basis of the pectoral fin flutter of embryonic Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. 48: 2223–2227.

The pectoral fin of embryonic Atlantic salmon (*Salmo salar*) flutters rhythmically through most of the early development, probably to promote water currents near embryonic respiratory surfaces. A descriptive mechanical model of the anatomical basis of the fin movements involves four components. The basal coraco-scapular complex serves as a fulcrum for fin movements. The actinost forms a thin vane, which is alternately abducted and adducted by two opposing muscle groups originating on the coraco-scapular cartilage and inserting distally on the actinost. The last component, the fin membrane, moves as a result of travelling waves passing distad as a result of flexion of the actinost — a "whiplash" type of motion. The first five spinal nerves may be involved in generation of the rhythmic flutter.

La nageoire pectorale de l'embryon du saumon de l'Atlantique (*Salmo salar*) s'agite de façon rythmée pendant tout le début du développement, probablement pour favoriser les courants d'eau près des surfaces respiratoires embryonnaires. Un modèle mécanico-anatomique rendant compte des mouvements de la nageoire comprend quatre éléments. Le complexe coraco-scapulaire basal sert de pivot à ces mouvements. L'actinost forme une mince pale tantôt tirée, tantôt poussée par deux groupes de muscles opposés ancrés au cartilage coraco-scapulaire et s'insérant distalement sur l'actinost. Le dernier élément, la membrane de la nageoire, suit les ondes qui passent distalement à la suite de la flexion de l'actinost, et bouge comme un fouet. Les cinq premiers nerfs de l'épine dorsale interviendraient dans ce mouvement.

Received January 22, 1991
Accepted May 15, 1991
(JA871)

Reçu le 22 janvier 1991
Accepté le 15 mai 1991

Tissues or organs may perform a different physiological role during embryogenesis than that performed in the postembryonic phase. In some species of salmonid fishes, the pectoral fin begins to flutter rhythmically shortly after its differentiation (well before hatching) and continues throughout most of the yolk resorption period (Stuart 1953; Peterson 1975). The function of this rhythmic flutter seems to be to generate water currents through the buccal cavity and/or over the anterior body integument to assist in delivering oxygen to respiratory surfaces. The frequency can be modified to some extent by varying the ambient oxygen concentration, but the dynamic range is not great, with the frequency near maximum even at oxygen concentrations of air saturation (Peterson 1975). It is temperature dependent (60–140/min over a range of 2–14°C, Peterson and Martin-Robichaud 1983), but is constant under constant conditions for about 180 degree-days (2 mo for these experiments.)

Thus far, embryos of Atlantic salmon (*Salmo salar*), brown trout (*S. trutta*), and perhaps brook trout (*Salvelinus fontinalis*) (White 1915) have been observed to show the pectoral fin flutter. We have not observed it with embryonic rainbow trout (*Oncorhynchus mykiss*), Atlantic cod (*Gadus morhua*), winter flounder (*Pseudopleuronectes americanus*), white sucker (*Catostomus commersonii*), or lumpfish (*Cyclopterus lumpus*) (unpublished observations).

In this paper, we reexamine the anatomy of the pectoral fin of embryonic Atlantic salmon and propose a mechanical basis for the fin flutter.

Materials and Methods

A series of Atlantic salmon embryos (Saint John River, N.B., origin, reared at 3–4°C) was dissected from the attached yolk sac, starting just before onset of the pectoral fin flutter and continuing weekly until terminal yolk resorption (80 d from start of sampling) (Table 1). Three embryos were placed in each of the appropriate fixatives (Bouin's for cresyl violet, 10% formalin for Kluver's and H&E, Bodian's fixative for Bodian's and Rager's) for five stains: haematoxylin–eosin for general tissue staining (Humason 1979), cresyl violet (Humason 1979), Kluver's cresyl violet stain (Kluver and Barrera 1953), Rager's (Rager et al. 1979), and Bodian's (Humason 1979). The last four are nervous tissue stains. After fixation, the embryos were embedded in paraffin and sectioned. One embryo was sectioned (10-μm sections) in each of three planes: transverse, coronal, and sagittal. In addition, some sections were made of the pectoral fin only and stained with cresyl violet.

Entire embryos were also cleared and stained for cartilage with alcian blue (Dingerkus and Uhler 1977). Drawings of fins and fin sections were made with the aid of camera lucida attachments and then retraced. Composite construction through the pectoral region was built up from serial camera lucida drawings on transparencies.

Results and Discussion

General Anatomy of the Pectoral Region of the Salmon Embryo

The pectoral fin of a newly hatched Atlantic salmon embryo is appressed to the body (Fig. 1). The opercula are not yet fully

TABLE 1. Timetable of events during embryonic development of Atlantic salmon (incubation temperature 3–4°C).

Days from fertilization	Approximate degree-days	Event
71	275	Median "eyed" stage
84	320	Initiation of pectoral flutter
122	425	Median hatch
141	490	Fin rays first discernible; pectoral flutter ceases soon after
164	580	Terminal yolk resorption

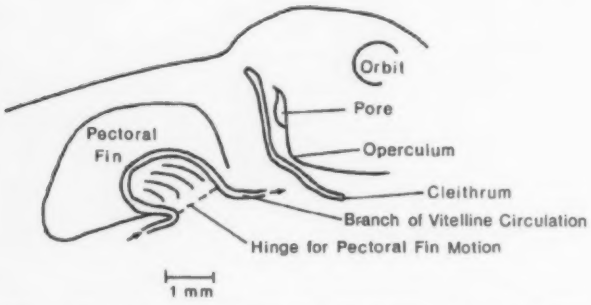

FIG. 1. Diagram of pectoral region of a newly hatched Atlantic salmon embryo. Arrows indicate direction of blood flow. Broken line indicates approximate location of the "glenoid line" where the actinost fuses with the coraco-scapular cartilage.

developed, and do not normally move. The fin's movement is hinged where it joins the body wall (broken line in Fig. 1). The fin is alternately abducted and adducted, with each phase beginning at the anterior fin margin and proceeding in a wave to the trailing edge. A "bailing" action is thus apparently produced which generates a pulsatile movement of water flowing posteriad from the back of the head (Peterson 1975). Water within the opercular cavity is drawn through a pore created between the posterior edge of the operculum and the cleithrum, the latter structure forming a ridge passing ventral onto the dorsal surface of the yolk sac.

Skeleton

The early skeletal development of the salmon pectoral fin has been elegantly described by Swinnerton (1905), and will be reviewed here with some further observations based on our histological results. In the earliest stages of pectoral fin development, the skeletal support is of two components: a distal blade-like cartilage (actinost) and a basal coraco-scapular cartilage (Fig. 2A, 2B). The actinost is thin and flexible, as observed from manual movement of the fin on whole embryos stained with alcian blue.

In the course of development, the actinost becomes increasingly perforated, and subsequently separated from the coraco-scapular cartilage as a series of actinosts — percursors of the radial bones (Fig. 2C). A series of distal knobs forms on these actinosts, the articulating surfaces for the developing fin rays. The upper distal cartilage (UDC), the articulating surface for the first ray, is apparently derived from the coraco-scapular cartilage in salmon. There is some disagreement on its origin in other species (Kohno and Taki 1983).

The basal, coraco-scapular cartilage, forming the fulcrum for the fin movement, is anchored in the body wall. As development proceeds, the cartilage elongates with posterior and dorsal coraco-scapular processes (Fig. 2A, 2B). These processes, plus the lengthened precursor of the coracoid bone, anchor the fin firmly. In addition, the coracoid, scapular, and dorsal coraco-scapular process all articulate with the cleithrum.

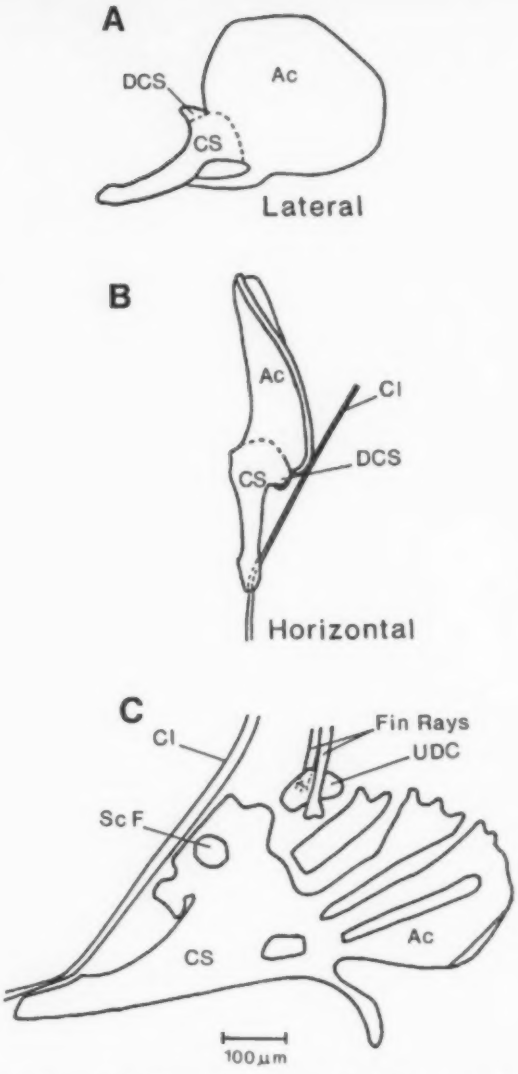

FIG. 2. Pectoral fin skeleton of an embryo 7 d after initiation of pectoral flutter, drawn from cleared and stained whole embryos. (A) Lateral view; (B) horizontal view, from above. Ac: actinost (blade-like cartilage); CS: coraco-scapular cartilage; DCS: dorsal process of coraco-scapular cartilage; Cl: cleithrum; broken line: glenoid line where the two elements of the girdle are fused. (C) Pectoral fin girdle (lateral view) of an embryo 2 mo older than the one illustrated in Fig. 3A and 3B. ScF: scapular foramen; UDC: uppermost distal cartilage.

Musculature

Pectoral flutter is generated by two opposing muscle groups (Fig. 3). The adductor muscle mass (adductors superficialis and profundus; Greene and Greene 1913; Winterbottom 1974)

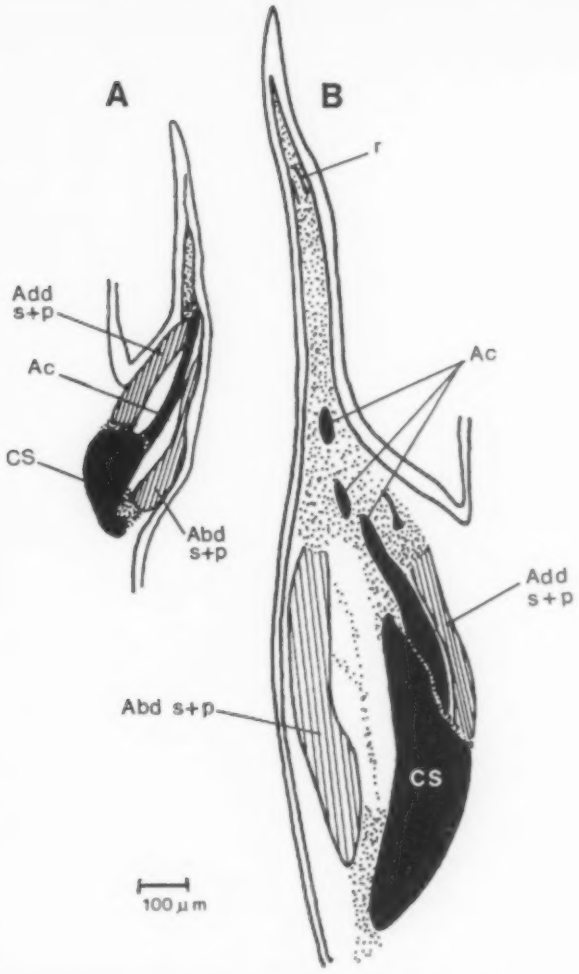

FIG. 3. (A) Cross-section through pectoral fin at initiation of pectoral fin flutter. Musculature hatched; cartilage solid; connective tissue stippled. Add s+p: adductors superficialis + profundus; Abd s+p: abductors superficialis + profundus; Ac: actinost; CS: coracoscapular. (B) Cross-section through pectoral fin of embryo 2 mo older than shown in Fig. 3A. Actinost has fragmented into radials and fin rays (r) are forming.

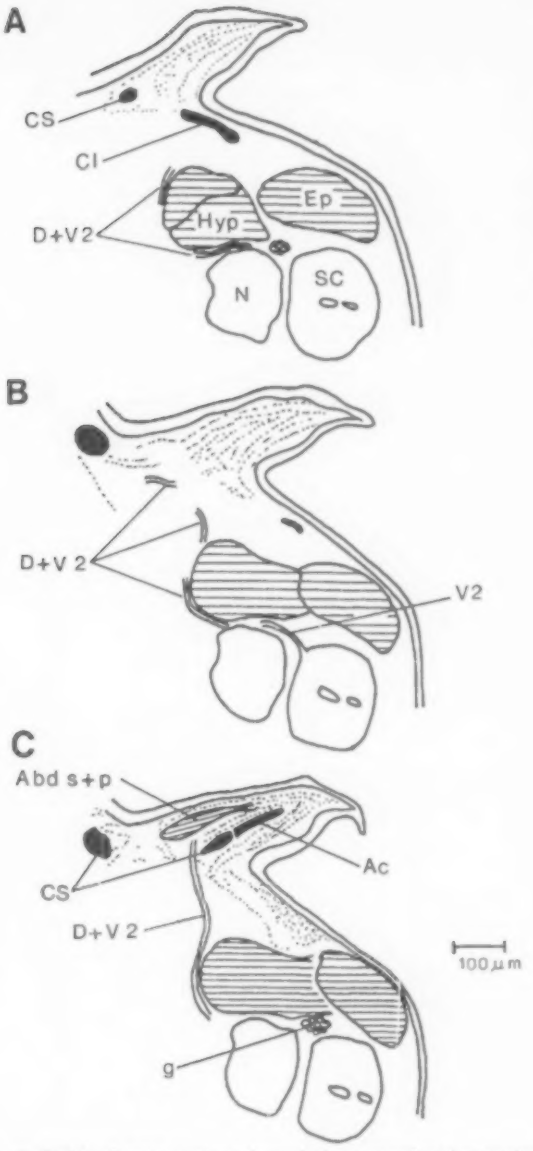

FIG. 4. Series of cross-sections through the pectoral region (stained with Bodian's) of a salmon alevin at initiation of pectoral fin flutter. A and B are one 10-µm section apart; B and C are consecutive sections. D+V2: dorsal plus ventral rami of spinal nerve 2, combined; V2: ventral ramus of spinal nerve 2; CS: coraco-scapular cartilage; Cl: cleithrum; SC: spinal cord; N: notochord; Ep: epaxial muscle; Hyp: hypaxial muscle; g: dorsal ganglion for spinal nerve 2.

draws the fin mediad, and the abductor muscles (superficialis and profundus) draw it laterad. Throughout most of the pectoral flutter phase (Table 1; Fig. 3A), fin rays have not differentiated, and the two opposing muscle groups originate on the coraco-scapular cartilage and insert on the outer edge of the actinost. The fin proper, at this early stage, consists of epidermis surrounding an interior substance resembling connective tissue (similar perhaps to the "squidge" of McCutchen 1970).

Toward the end of the fin flutter period (Fig. 3B), the muscle groups insert via tendons onto the separated actinosts plus the bases of the paired fin rays.

Innervation

The pectoral fin muscles are innervated well before initiation of the rhythmic flutter — probably during fin-bud formation (Géraudie 1985). By following nerve trajectories in embryo sections stained with Bodian's stain (Fig. 4), it was apparent that spinal nerves 1–5 could be involved in the innervation of the pectoral fin musculature. The dorsal and ventral rami join slightly ventral to the spinal cord, migrate ventrad between the notochord and epaxial muscle, and then continue mediad to the hypaxial muscles. Ventrolaterally of the hypaxial muscle mass, spinal nerves 1–3 form a plexus as do nerves 4 and 5 (Fig. 5). Branches from each of these plexi innervate both the adductor

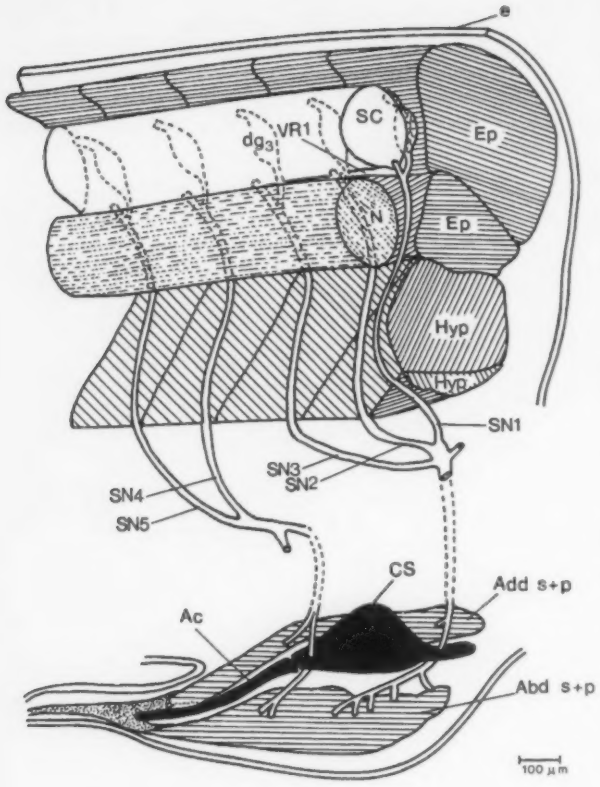

FIG. 5. Reconstruction of camera lucida drawings of sections through the pectoral region of an Atlantic salmon embryo at first fin movement. The perspective of the spinal cord and musculature is reconstructed from cross-sections, and that of the pectoral from fin horizontal sections. e: epithelium; dg_3: dorsal ganglion of spinal nerve 3; VR1: ventral ramus of spinal nerve 1; CS: coraco-scapular cartilage; Ac: actinost; Add s + p: adductor muscles superficialis plus profundus; Abd s + p: abductor muscles superficialis plus profundus; SC: spinal cord; N: notochord; Ep: epaxial muscle; Hyp: hypaxial muscle; SN1–5: spinal nerves 1–5.

and abductor muscles. To determine which of spinal nerves 1–5 send branches to the pectoral fin musculature from the plexi would require more elaborate tracer experiments as with the horseradish peroxidase technique.

Suggested Descriptive Model

We propose that the mechanics of the rhythmic pectoral flutter involves four components (Fig. 6). The first is the coraco-scapular complex with anterior and posterior extensions embedded as anchors in the body wall, which acts as a relatively massive base providing a fulcrum for fin movement. The second component is the actinost, a thin, stiff but flexible, skeletal vane connected to the base. The third component consists of the two opposing muscle groups which supply the power for the fin movements, and the fourth is the distal fin membrane acting as a whip-like structure.

The flexion of the actinost generates a travelling wave in the fin membrane. Alternate contraction of the opposing muscle masses flexes the thin actinost (= scapular-radial element) back and forth. This flexion, in turn, imparts an oscillatory flutter in the fin membrane. We also speculate that energetic efficiency may be increased if elastic energy is stored when the actinost is flexed and released during straightening, thus reducing the amount of work required during contraction of the opposing muscles.

Water is moved along the fin as the flutter is initiated at the anterior edge of the fin and proceeds posteriorly. This sequential activation of fin elements must be synchronized by sequential stimulation of muscle groups by the motor nerves innervating the fin. As many as five spinal nerves may innervate the pectoral fin, and sequential firing of motoneurones in these five nerves would be one way of achieving this synchrony.

The suggestion that the rhythmic fin flutter is under neuronal control also leads to the possibility that a rhythmic pattern generator, residing in the central nervous system, induces sequential stimulation of motoneurones. This pattern generator would seemingly have a transient existence, being "turned off" in some fashion when the fin flutter ceases. The origins of the pectoral fin flutter and its neuronal control are unknown, and are curious in that the flutter is not universally present in salmonids.

It is also apparent that the mechanism described above cannot be operative when the fin rays have developed sufficiently to

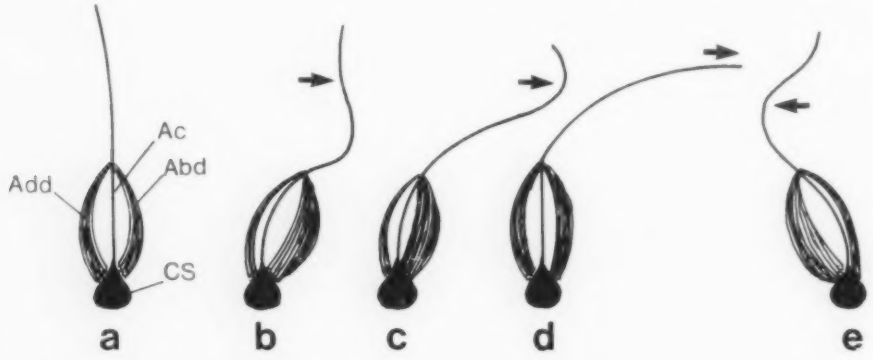

FIG. 6. Sequence of events illustrating the mechanism of the pectoral fin flutter. As a result of contraction of abductor muscles, a travelling wave is propelled along the fin membrane, as indicated by arrows in b, c, and d. In e, the opposing muscle group has contracted, initiating fin adduction.

impart a certain stiffness to the fin membrane. At this point, the fin musculature inserts via tendinous connections on the bases of the ray hemitrichia. Flexion of the fin is then probably achieved by alternate pulling on opposing hemitrichia as described by Arita (1971) for caudal fin flexion during swimming. By this stage of development the fin membrane is elongating and rotating into its final position to serve a stabilizer function. Further detailed observations relating cessation of pectoral flutter to fin ray formation would be useful.

The morphological changes in the pectoral fin toward the end of the embryonic period (change in orientation, formation of fin rays) with attendant changes in mechanics and function are adaptive. The embryonic fin is well designed to generate water currents around the alevin during an intragravel existence, while that of the postembryonic juvenile is essential to maintaining position near the substrate of swiftly flowing streams.

Acknowledgements

Drs. R. L. Saunders and J. Duston critically reviewed the manuscript. B. Best typed the manuscript and F. Cunningham aided in figure preparation.

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Effects of Incubation in Oiled Sediment on Emergence of Capelin (*Mallotus villosus*) Larvae

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Paine, M. D., W. C. Leggett, J. K. McRuer, and K. T. Frank. 1991. Effects of incubation in oiled sediment on emergence of capelin (*Mallotus villosus*) larvae. Can. J. Fish. Aquat. Sci. 48: 2228–2239.

Capelin (*Mallotus villosus*) were incubated in oiled sediment (range of initial concentrations 0–400 ppm total hydrocarbons) from the blastula stage to emergence of larvae. Concentrations of individual and total hydrocarbons in the sediment and interstitial water declined by ≈80–90% over the 28-d exposure period. Larvae from oiled sediment emerged ≈0.5–1 d earlier than did control larvae, and the percentage of larvae emerging was greater in the oiled treatments. Yolk length of exposed larvae after emergence was 10% greater than that of controls; head length (a measure of body size) was 1% less. The emergence behaviour of exposed larvae serves as an adaptive response to stress which enables more larvae to leave the contaminated sediment quickly. The yolk and body size effects would probably not affect survival. Therefore, oiled sediment in the tested range would not significantly affect embryos and larvae prior to emergence. Delayed effects on emerged larvae, which are more sensitive than embryos, may be more important.

Des capelans (*Mallotus villosus*) ont incubé dans des sédiments contenant au départ entre 0 et 400 ppm d'hydrocarbures totaux, du stade de la blastula à l'émergence des larves. Les concentrations d'hydrocarbures particuliers et totaux dans les sédiments et l'eau intersticielle ont diminué de 80 à 90 % environ durant la période d'exposition de 28 jours. Les larves des séminents ont émergé environ 0,5 à 1 jour plus tôt que les témoins et le taux d'émergence était plus élevé dans le premier cas. Le vitellus des larves exposées était de 10 % plus long que celui des témoins, tandis que la tête (une mesure corporelle) était plus courte de 1 %. Le comportement d'émergence des larves exposées est une réponse adaptative au stress qui permet à plus de larves de quitter les sédiments contaminés rapidement. Les effets sur le vitellus et la taille n'influent probablement pas sur la survie. Par conséquent, les sédiments contaminés aux concentrations étudiées n'agiraient pas de façon significative sur les embryons et les larves avant l'émergence. Les retards causés aux larves émergées, plus sensibles que les embryons, sont peut-être plus importants.

Received May 3, 1989
Accepted June 4, 1991
(JA157)

Reçu le 3 mai 1989
Accepté le 4 juin 1991

The recent development and expansion of offshore oil fields in the North Atlantic has led to concern about the effects of oil pollution from spills, blowouts, and chronic inputs on marine fisheries. This study continues an examination of the effects of Hibernia crude oil on capelin (*Mallotus villosus*) early life history stages (e.g. Paine et al. 1988). Capelin support an inshore fishery and are an important food source for other fish species such as cod (Bailey et al. 1977; Scott and Scott 1988). Most capelin stocks in the Northwest Atlantic spawn in intertidal or shallow subtidal areas; these are also areas where oil from spills or blowouts concentrates (Mann and Clark 1978). Oil pollution could therefore have a significant effect on capelin, as embryos and larvae are concentrated in large numbers on spawning beaches (Frank and Leggett 1984 gave an estimate of ≈10 billion eggs for a spawning beach 2400 m^2). Furthermore, abundance of 2-yr-old capelin is correlated with environmental conditions during their early stages (Leggett et al. 1984), suggesting that larval abundance may determine subsequent year-class strength.

Preliminary work focused on the effects of the water-soluble fraction (WSF) of Hibernia crude oil on capelin embryos and larve (Paine et al. 1988, 1992). Exposures comparable with those measured shortly after oil spills are lethal to larvae but not to embryos, and inhibit the growth of both stages. High concentrations of oil compounds dissolved in the water column do not persist because the most soluble compounds (mono- and diaromatics, low molecular weight aliphatics) are also the most volatile (Connell and Miller 1984). Thus, concentrations of hydrocarbons in the water column after a spill are not expected to affect capelin embryos and larvae, unless the spill occurs just prior to spawning and close to spawning grounds. However, oiling of sediments on spawning beaches represents a longer-term problem, as contamination can persist for >10 yr (Butler and Levy 1978). Hydrocarbons are exchanged between the sediment and interstitial water and could certainly affect capelin embryos incubating in contaminated sediment. This study examines the effects of incubation in oiled sediment on capelin embryos. A simple flow-through system for the exposures is described; few researchers have attempted to rear fish embryos in contaminated sediments, despite the growing recognition of

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the importance of sediments in assessing marine environmental degradation (Anderson 1988).

Materials and Methods

Study Site

The study was conducted in June and July 1988 in Bryant's Cove, Conception Bay, Newfoundland (47°40.5′N, 53°11.0′W; see Frank and Leggett 1981a, 1981b; Taggart and Leggett 1987a for site description). Capelin spawn in June on a gravel beach located at the head of the cove. Eggs are adhesive and incubate in the inter- and subtidal sediments. Embryos hatch after 2–3 wk and larvae may remain in the gravel for a few days after hatch. Larvae usually emerge from the sediments during onshore winds. These winds bring warm, food-rich, predator-poor water inshore, and emergence during onshore winds appears to be an active, adaptive response (Frank and Leggett 1981a, 1981b, 1983). In this study we focus on the effects of oiled sediment on the timing of larval emergence, and on the condition and size of emerged larvae.

Experimental System and Procedures

Capelin embryos were exposed continuously from the blastula stage through to emergence of larvae to six treatments or nominal concentrations of oiled sediment: 0, 25, 50, 100, 200, and 400 ppm total hydrocarbons. These concentrations spanned the lower end of the 100–1000 ppm range observed on contaminated beaches (Pinto et al. 1984). Embryos were reared in a flow-through system similar to that used by Frank and Leggett (1983), designed to simulate natural temperature conditions in low tide or subtidal areas. Four 40-L aquaria were each divided into six compartments (one per concentration) by glass dividers attached to the aquarium sides and bottom with silicone sealant. Seawater was pumped from a site ≈200 m from shore. The incoming flow was filtered and then split, with one line providing water for the bath holding the aquaria and the other line providing water to a 20-L reservoir. Water from the reservoir supplied individual compartments in the aquaria via gravity flow.

The flow rate to each compartment was 50–70 mL/min or 3.6 L/h and was regulated by adjusting plastic clamps on the inflow tubes. The volume of water in each compartment was ≈3 L; the flow rate provided that volume every 50 min. The flushing rate in our experimental system was much greater than that in Bryant's Cove, as 100% of the cove volume is advected out of the cove every 15–19 h (Taggart and Leggett 1987b, 1987c). A flow rate of 3–4 mL/min would have been more realistic, but would have increased residence times and temperatures of water in the siphon hoses and compartments beyond acceptable levels.

An undergravel filter system was set up in each compartment to ensure that incoming water flowed down through the gravel before leaving the compartment (Fig. 1). Water entering the compartment was drawn through the gravel and the slotted filter base and then out of the aquarium via the glass siphon tube. The compartments contained 6 cm of gravel: 2 cm of egg-free gravel, 2 cm of egg-bearing gravel, and 2 cm of oiled gravel. Egg-free gravel was collected from the beach prior to capelin spawning, washed, and then dried in the sun. Most of the fine (<1 mm) fraction was removed by sieving; rocks and pebbles >10 mm were also removed. The final composition by volume, measured at the end of the experiment, was 36% >5.6 mm, 47% 2–5.6 mm, 11% 1–2 mm, and 6% <1 mm. The contribution of the <1 mm fraction was underestimated because fine sediment was lost through rinsing during the recovery of embryos and larvae at the end of the experiment.

Egg-bearing gravel was collected from the intertidal on the afternoon of 17 June 1988. The gravel was rinsed in a 1-mm sieve to remove fine sediment. Rocks and pebbles >10 mm were also removed and then the remaining gravel was added to aquaria. The eggs resulted from subtidal spawning on the night of 16 June, and early morning of 17 June, when large numbers of capelin were observed nearshore. Four samples of 23–44 eggs were examined to age the embryos. Ninety percent were high blastulae, indicating that they were <1 d old. The remainder were dead, damaged, or unfertilized; no older stages were observed. All times are given as days from 00:00 17 June which approximates the age of embryos from fertilization.

Hibernia crude oil was obtained from Mobil Canada Ltd. in St. John's, Newfoundland. The oil was provided in sealed drums lined with teflon. On receipt, the oil was transferred to 4-L glass jars, which were then sealed and stored at 4°C. Oiled gravel was prepared by adding 12 times the amount of oil required to produce the nominal concentration to the appropriate quantity of egg-free gravel. Trials conducted in 1987, and those of Pinto et al. (1984), indicated that 25% of the oil would be retained in the gravel. This provided triple the nominal concentration in the top layer of oiled gravel, which accounted for one third of the total gravel volume. Oiled gravel was prepared by adding the oil to a small quantity of gravel, shaking the mixture thoroughly in a glass jar, and then repeatedly adding more gravel and shaking the resultant mixture. The oiled gravel was then rinsed and added over the egg-bearing gravel.

Embryos were incubated below, rather than in, oiled gravel for three reasons. First, we did not want to risk damaging embryos by mixing (shaking) oil and egg-bearing gravel. Second, with embryos incubated at depths up to 12 cm under natural conditions (Frank and Leggett 1981b), most embryos would not be in an oiled surface layer. Third, we wanted larvae to emerge through an oiled layer to determine if there was any behavioural avoidance of contaminated sediment which might prevent or restrict emergence.

A 16 h light : 8 h dark photoperiod was maintained throughout the experiment. Flow rates were adjusted every 3–4 d. Visible oil slicks formed on the surface in some compartments during the first few days. These were removed with paper towel. One aquarium (aquarium 4) was sampled to measure embryonic mortality at ages 1, 3, 7, and 15 d. Approximately 75 mL of gravel was removed from each compartment with a plastic scoop and preserved in 10% $CaCO_3$-buffered formalin. Dead embryos were not distinguishable from live developing embryos in the early samples because the KOH separation used to break the adhesive bond between the eggs and the gravel damaged the embryos. At age 15 d, embryos were well into organogenesis, with pigmented and visible eyes, and embryos that had died during earlier stages could be identified easily.

When emergence began, emerged larvae were removed regularly. Several sweeps were made through each compartment with a nylon mesh net having the same width and height as the water column until few or no larvae were caught. Initially (starting 5 July or age 18 d), larvae were collected every 6 h starting at 03:00. Beginning 21:00 7 July (20 d), larvae were collected twice daily, at 09:00 and 21:00. Sampling frequency was reduced because we could not collect larvae quickly enough to sample more frequently. Beginning 11 July (24 d), larvae were

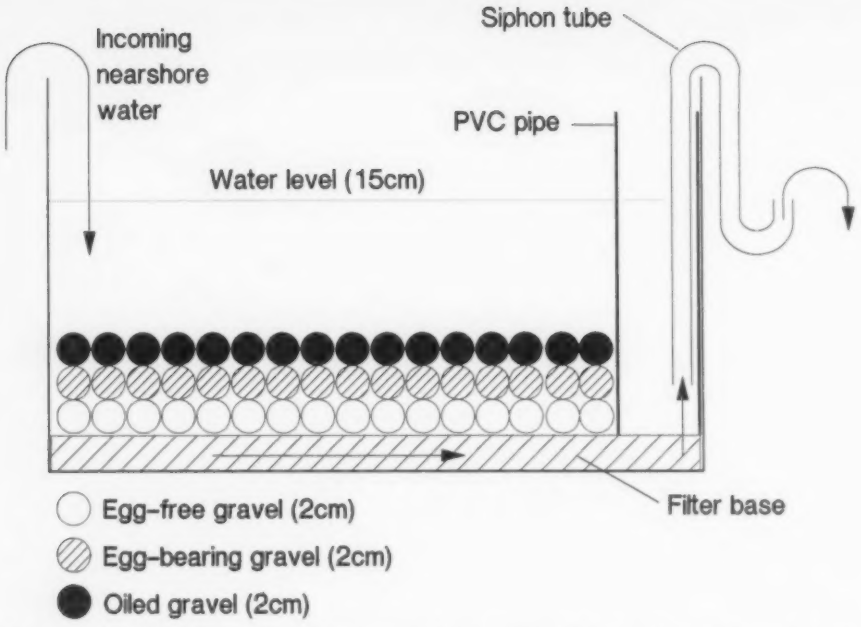

FIG. 1. Design of compartments and arrangement of gravel layers in the experimental system. Arrows indicate direction of water flow.

collected at 07:00 and 19:00 to prevent the evening collection from extending beyond 22:00, when the lights went out.

The experiment ended following the morning collection of 14 July (27 d). Most of the water was siphoned from the compartments via the PVC pipe, and the filters and gravel were then removed. Larvae or eggs remaining in the compartments were removed with a net. The gravel from each compartment was placed in a bowl of seawater, the bowl was swirled, and the water was then poured through a 100- to 180-μm sieve. This procedure, which recovered most of the unemerged larvae, was repeated several times, either immediately or several weeks later, when the preserved gravel sample was soaked in 2% KOH for 24–36 h (see Taggart and Nakashima 1987). The eggs and any remaining larvae were recovered after KOH treatment by repeatedly adding water to the gravel, stirring, and passing the water through a 180-μm sieve.

All larvae and embryos were preserved in 10% buffered formalin. The larvae and embryos from each collection (during emergence or at the end of the experiment) were counted. The beaker method (Van Guelpen et al. 1982) was used to subsample when numbers were >2000. Head length (after Snyder et al. 1977) and yolk length were measured on a random sample of 10 larvae from each collection of emerged larvae and 20 unemerged larvae from the final collections. Each counted collection or subsample of >50 emerged larvae was dried overnight at 60°C and weighed to provide dry weights.

Statistical Analysis

For all analyses, the experimental design was a randomized complete block, with aquaria as blocks and oil concentration as the treatment. Individual observations were data from each compartment. The concentration effect was subdivied using two orthogonal contrasts (Sokal and Rohlf 1981, p. 232–242): controls versus oil concentrations and among oil concentrations. These contrasts provided an indication of how much of the oil effect was due simply to the presence of oil (first contrast) and how much was due to the strength of the dose (second contrast). Differences were considered significant when $P \leq 0.05$. All analyses were conducted using SAS statistical packages (SAS Institute Inc. 1987).

Counts of the total number of emerged larvae, unemerged larvae, and unhatched embryos were available for each compartment. Counts from aquarium 4 were corrected for the 100-mL gravel samples removed for chemical analysis (see below) just before the aquaria were emptied. We assumed that these samples contained unemerged larvae and unhatched embryos at the same densities as in the remaining gravel. This correction was only required for the one set of samples taken after emergence began. These samples contained unemerged larvae and unhatched embryos which were not counted, but the larvae emerging from the gravel had been counted. This bias would not arise for samples taken prior to emergence.

Hatching success and emergence were assessed by analyzing percent hatch (emerged and unemerged larvae as a percentage of the total number of larvae and embryos) and percent emergence (the percentage of larvae emerging). The timing of emergence was examined by developing two variables from counts of larvae emerging on 15 collection dates. The counts, expressed as percentages of the total number of emerged larvae, were subjected to principal components (PC) analysis. The covariance matrix, rather than the correlation matrix, was factored so that the PCs reflected absolute variance on the original scale. The PC analysis was an objective means of creating two independent variables (PCs) from 15 original correlated variables.

Effects on size were assessed by examining several variables based on head and yolk length and dry weight. Overall mean

dry weight was the mean dry weight of larvae emerging from 21 to 27 d and was calculated by weighting the mean dry weight for each collection period by the number of larvae emerging at that time. Twenty-two of 336 dry weights were estimated from regressions of dry weight on yolk and head length, because there were too few (<50) larvae to weigh or because the measured weights were obvious measurement errors.

Four derived variables were calculated using head length measurements: (1) OVERALL = grand mean head length of emerged larvae, weighted according to number emerged in each collection period; (2) MEAN = mean of collection period means (reflects size at fixed age, as it was not weighted by numbers emerging); (3) NIGHT/DAY = mean of mean head lengths from morning collections minus mean of means from evening collections (reflects diel size differences); (4) END = mean head length of unemerged larvae.

Five derived variables were calculated from yolk lengths (log-transformed prior to calculations): (1) OVERALL = calculated as for head length; (2) MEAN = calculated as for head length; (3) SLOPE = slope of the regression of log yolk length on age, in days (reflects developmental and growth rates); (4) NIGHT/DAY = the mean of residuals from the yolk length-age regression for morning collections minus the mean of residuals for evening collections (reflects diel size differences); (5) END = calculated as for head length.

Chemical Analyses

Concentrations of total and specific hydrocarbons were measured on gravel and interstitial water samples; concentrations of specific compounds were also measured on an oil sample. Samples (100 mL) of each concentration of oiled gravel were taken immediately after mixing, and from aquarium 4 after 7 and 27 d. The samples were taken with a plastic scoop, through the entire 6-cm gravel layer. Interstitial water samples were taken after 1, 3, 7, 15, and 27 d from the 0, 100, and 400 ppm treatments. Each sample consisted of 300 mL per concentration from each of aquaria 1–3. The water was siphoned slowly from the PVC pipe and therefore represented interstitial water plus some water from just above the substrate (there were <300 mL of water in the interstices and below the filter base, and water for the samples was siphoned at a greater rate than the incoming flow rate).

Total hydrocarbon concentrations in the gravel and water samples were determined by ultraviolet (UV) spectrophotometry (Mackay and Szeto 1981). Hydrocarbons were extracted from water samples using dichloromethane. Gravel samples were dried overnight at 40°C and then subjected to Soxhlet extraction for 24 h using dichloromethane. Concentrations were determined by measuring the absorbance at 231 nm and converting that to hydrocarbon concentrations using a standard curve developed from successive dilutions of the crude oil in dichloromethane. This method provides a measure of the oil content (total hydrocarbons) of the samples, provided that contamination from other hydrocarbons is negligible.

Concentrations of individual aromatics and aliphatics were determined by gas chromatography. Dichloromethane extracts were separated on a silica gel. The aliphatic fraction was eluted with hexane and the aromatic fraction with dichloromethane. The fractions were evaporated to a volume of 0.5 mL, and samples of 1.2 μL were injected into a fused silica capillary column with helium as the carrier gas. Peaks on the resulting chromatographs were compared with those generated by standard solutions of known concentrations of specific aliphatics or aromatics. Although a large number of compounds were assayed, we restrict our discussion of the results to four broad groups: odd and even C8–C26 *n*-alkanes, naphthalenes (naphthalene, 1- and 2-methyl naphthalene), and other polycyclic hydrocarbons (PAHs). Monoaromatics were present in gravel and water, but those which were identified made a negligible contribution.

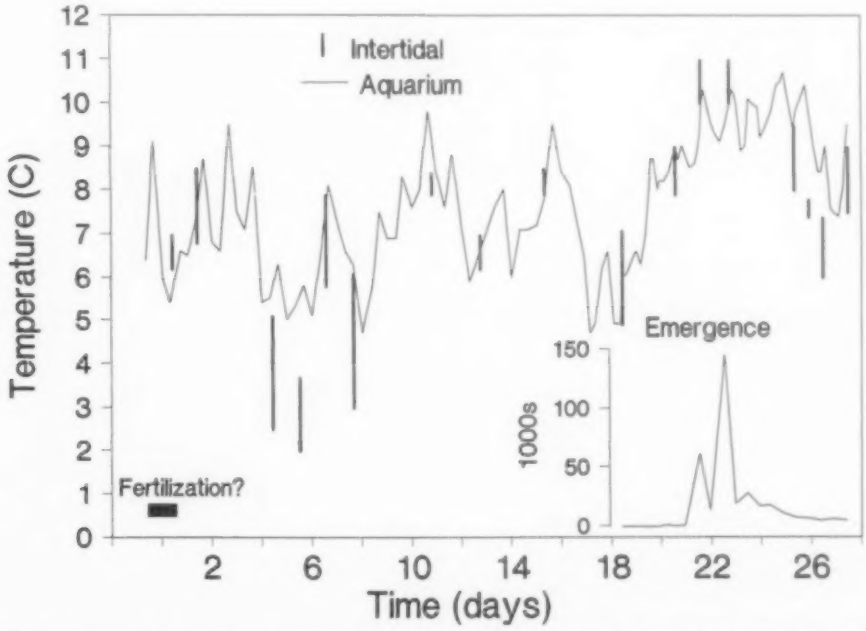

FIG. 2. Intertidal and aquarium temperatures, and total numbers of larvae emerging per collection period.

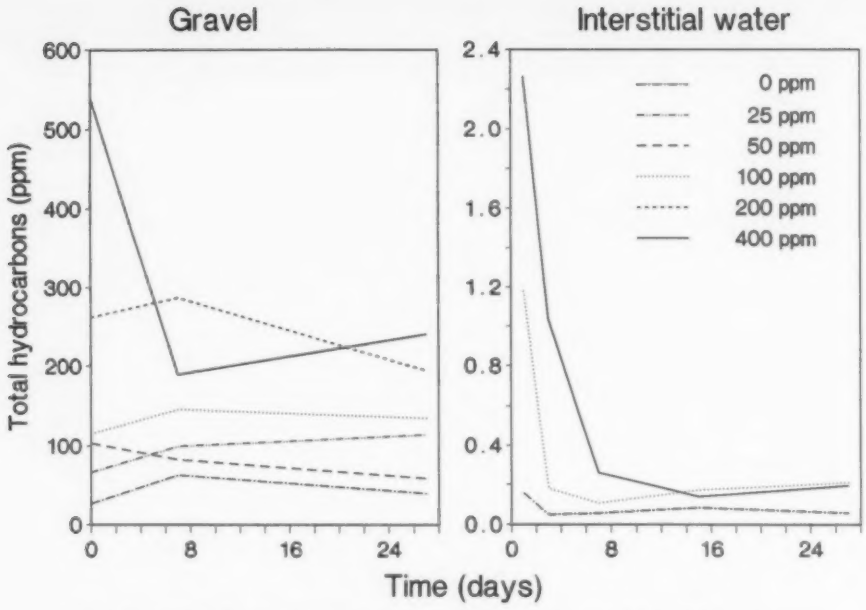

FIG. 3. Changes in total hydrocarbon concentrations in gravel and interstitial water.

TABLE 1. Composition of oil, gravel, and water samples. ND = not detected.

Sample	Naphthalenes[a]	Other PAHs[b]	*n*-Alkanes (C8-C26) Odd	Even	Total	Odd/even[c]
Hibernia crude oil (ppm)	2100	700	21 400	23 600	44 900	0.906
440 ppm gravel (ppb)						
0 d	300	320	11 100	11 200	22 300	0.992
7 d	90	110	4 100	4 300	8 500	0.948
27 d	170	60	600	700	1 300	0.790
400 ppm water (ppb)						
1 d	7.4	ND	17.4	21.0	38.4	0.833
7 d	ND	0.3	0.4	0.5	0.9	0.673
27 d	0.7	0.8	0.2	10.8	11.0	0.023
Control water (ppb)						
1 d	0.8	ND	0.5	8.2	8.7	0.056

[a]Includes naphthalene, 1- and 2-methyl naphthalene.
[b]Includes acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene. Measured but not detected in oil, water, or gravel were chrysene, benzo[*a*]anthracene, perylene.
[c]With 10 even compounds and 9 odd, values would be 0.9 if all concentrations were similar.

Our list of PAHs was incomplete, restricted to unsubstituted compounds (except for the methyl naphthalenes). However, there were no obvious unidentified peaks on the chromatographs that suggested high concentrations of an unknown high molecular weight compound.

Results

Physical

The system successfully reproduced the thermal regime of the inter- and subtidal zones. Water temperature was monitored continuously in aquarium 3 and was, on average, 0.7°C ($n = 16$, SD = 1.2) above the midpoint of temperatures recorded at the same time during high tide in the intertidal (Fig. 2). The biggest differences occurred early in the experiment when the laboratory system could not reproduce field temperatures in the 2–5°C range. Other aquaria were 0–0.5°C cooler than aquarium 3; compartments within an aquarium also differed by up to 0.5°C. Water temperature in the compartments and siphon hoses was affected by air temperature. Note the daytime peaks and nighttime troughs in the temperature record in Fig. 2. We have no comparable data on diurnal patterns for the intertidal.

Chemical

Changes in concentrations of total hydrocarbons in gravel and water are shown in Fig. 3. At mix, actual concentrations were very close to nominal concentrations. Concentrations measured from aquarium samples were expected to be more

TABLE 2. Final counts of emerged and unemerged larvae and unhatched embryos for the 24 compartments. Initial egg densities were calculated from these counts; densities in parentheses for aquarium 4 were from samples. Gravel volume per compartment was 1200 cm^3 for aquaria 1–3 and 820–890 cm^3 for aquarium 4 because of gravel removed in samples (see text for explanation). Numbers of unemerged larvae and unhatched embryos in aquarium 4 were adjusted for the loss in samples taken at age 27 d.

Aquarium	Nominal concentration (ppm)	Larvae		Embryos	Total	Egg density (no·/cm^3)
		Emerged	Unemerged			
1	0	13 149	12 020	716	25 885	21.6
	25	21 803	7 056	1 492	30 351	25.3
	50	14 756	4 132	4 388	23 276	19.4
	100	16 673	4 936	2 692	24 301	20.3
	200	6 131	3 701	866	10 707	8.9
	400	22 770	6 060	2 420	31 250	26.0
2	0	15 705	10 880	3224	29 809	24.8
	25	14 357	4 668	4660	23 685	19.7
	50	11 194	4 432	5316	20 942	17.5
	100	15 906	1 648	3124	20 678	17.2
	200	19 807	3 816	1316	24 939	20.8
	400	10 887	2 788	780	14 455	12.1
3	0	9 968	12 136	3156	25 260	21.1
	25	10 124	6 000	5504	21 628	18.0
	50	13 963	8 360	8556	30 879	25.7
	100	4 187	5 064	3652	12 903	10.8
	200	12 346	6 260	1552	20 158	16.8
	400	10 894	3 000	1722	15 616	13.0
4	0	16 494	2 275	213	18 982	23.2 (20.9)
	25	14 250	1 913	186	16 349	18.8 (18.5)
	50	23 164	4 228	517	27 909	32.1 (27.3)
	100	11 605	1 189	147	12 941	14.9 (16.9)
	200	20 256	1 842	202	22 301	25.1 (26.1)
	400	16 842	2 363	396	19 601	22.2 (24.5)

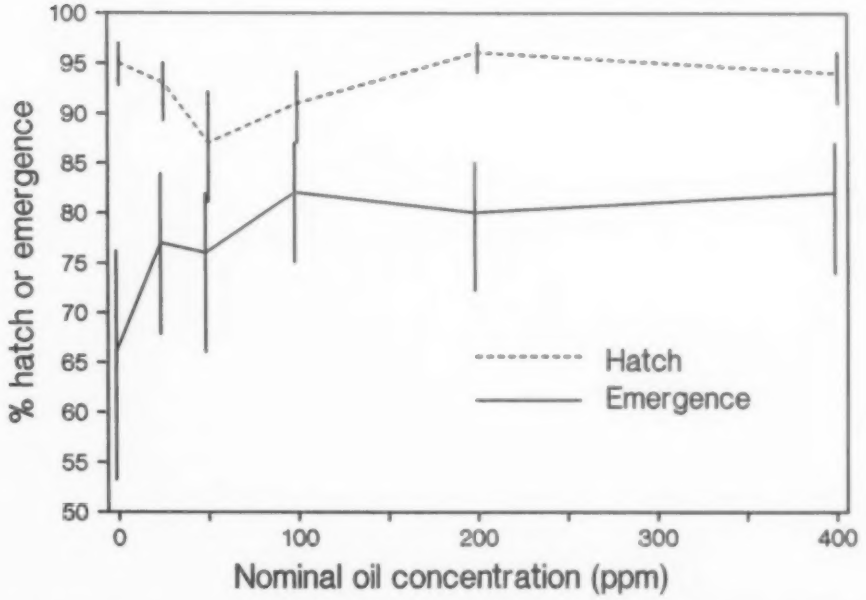

FIG. 4. Hatch and emergence. Percent hatch is the percent of embryos that hatched; percent emergence is the percent of hatched individuals that emerged. Values are means ± 2 SE, back-transformed from arcsine square-root.

TABLE 3. Correlations (r) of emergence counts (as % of total emerged larvae) from 15 collection periods with principal components (PCs) derived from factoring the covariance matrix. $|r| \geq 0.4$ are underscored.

Collection period (days after fertilization)	Mean ± SD	Correlation with: PC1	PC2
≤ 20 (a.m.)	0.5 ± 0.5	<u>−0.40</u>	<u>0.40</u>
20 (p.m.)	0.2 ± 0.1	−0.39	0.25
21 (a.m.)	17.2 ± 10.0	<u>−0.88</u>	<u>0.47</u>
21 (p.m.)	4.0 ± 2.1	−0.29	0.16
22 (a.m.)	40.5 ± 9.9	<u>0.82</u>	<u>0.57</u>
22 (p.m.)	5.7 ± 2.7	−0.01	<u>−0.47</u>
23 (a.m.)	8.5 ± 3.6	<u>0.48</u>	<u>−0.65</u>
23 (p.m.)	5.3 ± 2.0	−0.06	<u>−0.68</u>
24 (a.m.)	5.2 ± 2.6	<u>0.45</u>	<u>−0.49</u>
24 (p.m.)	3.5 ± 1.9	−0.32	<u>−0.83</u>
25 (a.m.)	2.3 ± 1.2	−0.28	<u>−0.83</u>
25 (p.m.)	2.1 ± 0.9	−0.27	<u>−0.79</u>
26 (a.m.)	1.4 ± 0.6	−0.22	<u>−0.69</u>
26 (p.m.)	2.0 ± 1.3	0.16	<u>−0.52</u>
27 (a.m.)	1.4 ± 0.6	−0.04	<u>−0.65</u>
% variance		61	29

variable because the distribution of the oil was more heterogeneous than in freshly mixed samples. Concentrations declined very little in the 25–200 ppm treatments, but declined by 60–70% in the 400 ppm treatment during the first week. As a result, concentrations converged, reducing the original 16-fold range. However, ranks of measured concentrations generally matched those of nominal concentrations. There may have been an upper limit to the amount of oil which could be sorbed by the gravel, as the fine (<1 mm) fraction was removed prior to the experiment. This fraction was probably more organic than larger fractions, and sorbance should increase with increasing organic carbon content of particles (and with decreasing particle size) (Connell and Miller 1984).

Total hydrocarbon concentrations in the interstitial (outflow) water declined from 1–2 ppm after the first day to 0.1–0.2 ppm after 3 (100 ppm treatment) to 7 (400 ppm treatment) d. Hydrocarbon concentrations in the interstitial water declined more rapidly at the lower concentrations (i.e. the removal rate was slower), consistent with the observed convergence of gravel concentrations. Note that the background level in the interstitial water from the 0 ppm treatment (control) was elevated on the first day. This probably reflected the contribution of plasticizers and other compounds from the filter base, although the bases had been left in flowing water for a week prior to the experiment to remove these compounds.

Relative to the parent oil, oiled sediment (400 ppm treatment) was enriched in aliphatics and other PAHs and deficient in naphthalenes (Table 1). Over the 27 d of the experiment, concentrations of *n*-alkanes in the gravel were reduced by ≈95%. The decrease in concentrations of the aromatics was less (50 or 70–80%, depending on how the increase in naphthalenes between 7 and 21 d is interpreted), although concentrations were lower and therefore less precise. The decrease in total hydrocarbon concentration in the 400 ppm treatment over 27 d was 87%, similar to the estimates obtained for specific compounds. Concentrations of the specific groups in the interstitial water, and in the gravel, were higher after 0 or 1 d than after 7 d, indicating that most of the hydrocarbons were removed early in the experiment. Again, this is consistent with the data for total hydrocarbons. Finally, note the presence of contamination, probably from the filter base or other plastics, in the control (0 ppm) interstitial water and in the interstitial

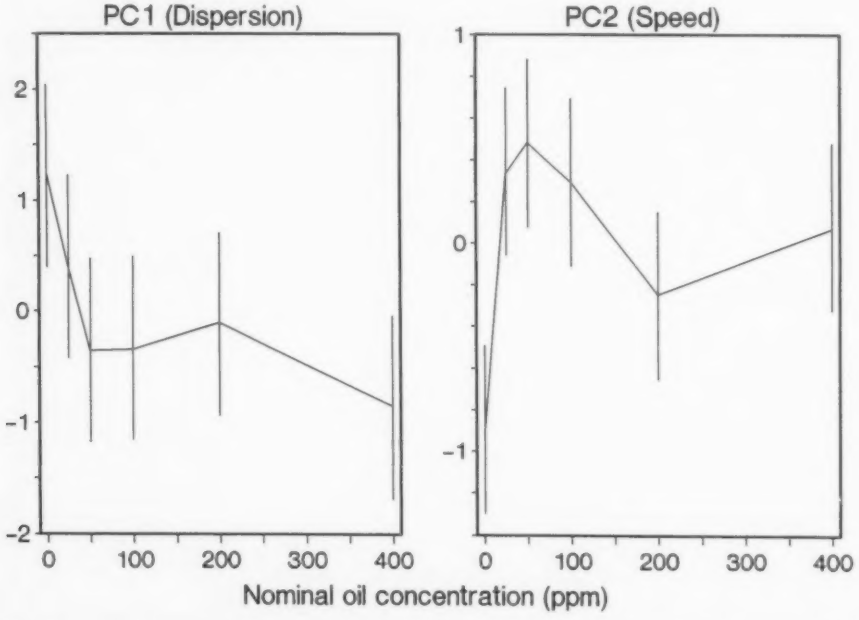

FIG. 5. Principal component (PC) scores. High values of PC1 indicate that emergence was concentrated around 22 d; low values indicate greater dispersion of emergence. Higher values of PC2 indicate earlier emergence. Values are means ± 2 SE.

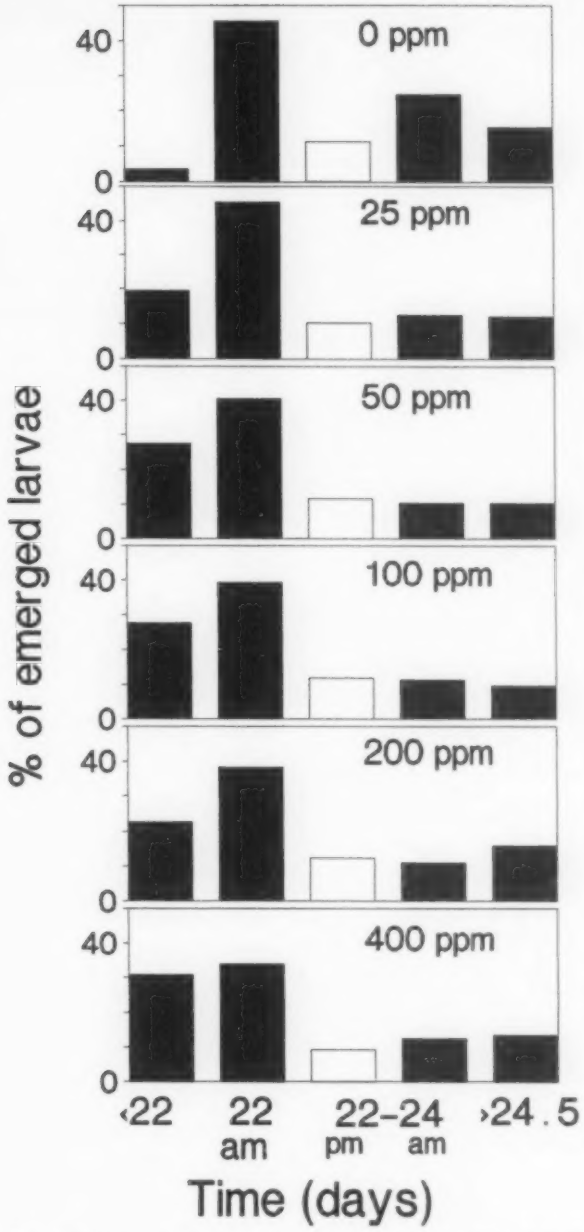

FIG. 6. Emergence patterns for each concentration. For 22 p.m. to 24 a.m., dark bars are overnight emergence (morning collections) and clear bars are daytime emergence (evening collections).

water from the 400 ppm treatment taken at 27 d. The non-oil contaminants decreased the odd to even ratio for *n*-alkanes.

Biological

Numbers of unhatched embryos, unemerged larvae, and emerged larvae are given for each compartment in Table 2. Initial densities ranged from 9 to 32 eggs/cm^3 of gravel. If all eggs remained in the 2-cm egg-bearing layer, densities in that layer would have been 27–96 eggs/cm^3. This compares favourably with the range of 10–140 eggs/cm^3 prior to emergence reported by Frank and Leggett (1981b) for the beach at Bryant's Cove.

Mortalities in samples taken at age 15 d were ≤2%, suggesting that egg loss prior to emergence was minimal. However, it is possible that embryos dying prior to that decomposed, in which case they would not be recorded as mortalities in later samples. If decomposition occurred, egg densities in the samples taken at 1, 3, 7, and 15 d would have declined with time; they did not (Page's test for ordered alternatives, $L = 154$, $P > 0.05$; Sokal and Rohlf 1981, p. 547). Final densities in the compartments were also not lower than densities in the samples (Table 2), although we probably would not be able to detect the loss of <1000–5000 eggs.

The first larvae to emerge were collected on the morning of 5 July (18 d), although >10 per compartment were not collected until the morning of 7 July (20 d). Emergence was coincident with a rise in temperature resulting from the effect of onshore winds on the nearshore water mass. Emergence peaked on the mornings of 8 July (21 d) and 9 July (22 d) (Fig. 2). Survival in the system was high, with 70–99% of embryos hatching. Most (90% in aquaria 1–3, 70% in aquarium 4) of the unhatched embryos remaining after 27 d were at an advanced stage of development, indicating that they did not die early in the experiment, and that they probably would have hatched. Hatching success and percent emergence were higher in aquarium 4 than in other aquaria (Table 2). Percent hatch differed significantly ($P \leq 0.05$) among oil concentrations and was lowest at intermediate concentrations (Fig. 4). Percent emergence did not differ significantly among all treatments, or among oiled treatments, but was significantly lower in controls (Fig. 4). There was a weak tendency for percent emergence to increase with increasing oil concentration.

The two PCs derived from factoring the covariance matrix of emergence data from 15 collection periods are described in Table 3. The first PC was positively correlated with overnight emergence at ages 22–24 d and negatively correlated with emergence at most other times. This PC is therefore a measure of the dispersion of emergence, with positive values indicating that emergence was concentrated at ages 22–24 d and negative values indicating that emergence was more evenly spread over the collection periods. Note that the morning, but not evening, collections at ages 22–24 d were emphasized. PC1 scores differed significantly among treatments, with most of the difference occurring between controls and oiled treatments (Fig. 5). Emergence of control larvae was concentrated at ages 22–24 d whereas emergence was more evenly distributed in other treatments (Fig. 6). The strong day–night difference observed at ages 23 and 24 d in the controls was not evident in the oiled treatments.

The second PC was positively correlated with early emergence and negatively correlated with later emergence (Table 3). Positive values indicated early emergence and negative values delayed emergence. PC2 scores differed significantly between controls and oiled treatments, as control larvae emerged later than exposed larvae (Fig. 5, 6). There was no further obvious relationship between concentration and PC2 scores.

Growth

Head length of emerged larvae followed a parabolic relationship with time, reaching a maximum coincident with peak emergence at ages 21 and 22 d (Fig. 7). There was a clear diur-

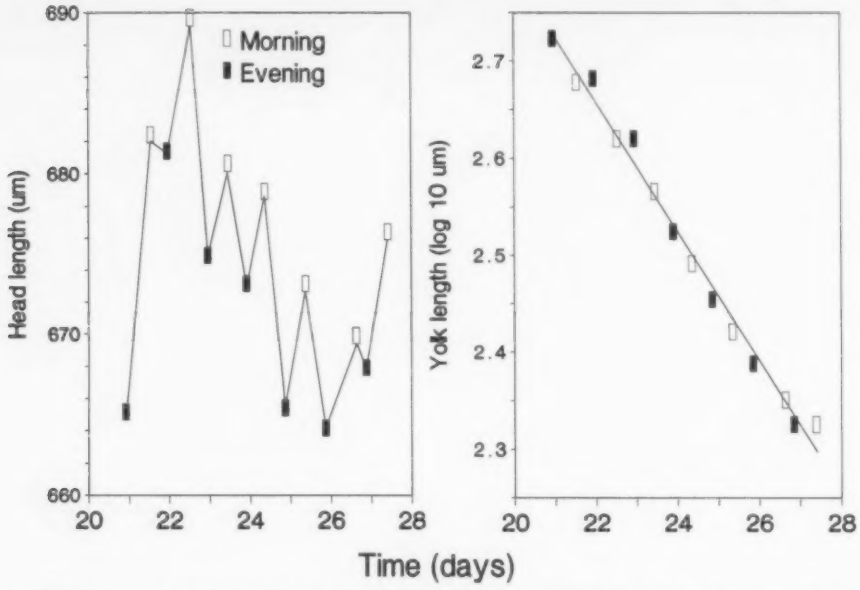

FIG. 7. Changes in head and yolk length (means for all compartments) with time. The regression equation for yolk length is log (yolk length) = 1.1234 − 0.0666 (days) ($n = 14, r^2 = 0.991, P < 0.0001$).

TABLE 4. Mean values for head length variables (μm) and *F*-values from ANOVAs testing for differences among concentrations. $^*P \leq 0.05$.

	Variable			
	Overall	Mean	Night/day	End
Nominal concentration (ppm)				
0	687	676	−12	662
75	686	675	−8	673
150	685	671	−9	662
300	678	672	−5	668
600	680	670	−6	657
1200	678	669	−9	650
SE	3	2	2	6
F				
Concentration	2.0	2.2	1.0	2.1
Oil versus control	3.1	5.2*	3.0	0.0
Among oil	1.7	1.4	0.5	2.6

nal difference in the size of emerged larvae. Larvae collected at night had heads ≈8 μm shorter than those of larvae collected on the following morning. This was not entirely a function of overnight growth, as head lengths of larvae collected at night were often less than those from larvae collected the preceeding morning. Larvae with larger heads were apparently more likely to emerge overnight than were larvae with smaller heads. The reason for this phenomenon is unknown.

Results of ANOVAs examining head length are given in Table 4. The only significant difference in 12 tests was between controls and oiled treatments for MEAN (head length at fixed age). The difference between controls and 1200 ppm, the treatment with the smallest larvae, was 7.7 μm, or only 1.1% of the mean over all compartments.

Yolk lengths (log-transformed) of emerged larvae declined almost linearly with time, with diurnal differences apparent only at earlier ages (Fig. 7). Exposure to oiled sediment clearly affected yolk size (Table 5). Effects on SLOPE and END were not significant; effects on NIGHT/DAY were greater, but also not significant. There was a weak tendency for diurnal differences to increase with increasing oil concentration. OVERALL and MEAN both differed significantly among treatments. Yolk length increased with increasing concentration (Table 5). The effects on OVERALL were stronger than those on MEAN, because the former included effects on time of emergence, as well as effects on yolk length at fixed ages indicated by the latter.

Dry weights (log-transformed) of emerged larvae declined linearly with age, as did yolk length. This suggests that yolk size variation contributed more to weight variation than did head length (body size) variation. Correlations among the three variables, and regressions predicting weights from yolk and head length, confirmed this interpretation. However, oil effects on overall mean dry weight of emerged larvae from ages 21 to 27 d were not as strong as those on yolk length, and only the differences between controls and oiled treatments were significant (Fig. 8).

Discussion

Oiled sediment affected hatching, emergence, and size of emerged larvae. The parabolic relationship between percent hatch and oil concentration makes it difficult to apply those results to field situations. Exposed larvae emerged 0.5–1 d earlier than did controls, and emergence was more evenly spread over time. There were also subtle alterations of diurnal patterns in oiled treatments. The net result was that more larvae emerged from oiled gravel than from control gravel. We conclude that accelerated emergence is a means of escaping stressful conditions. Exposed larvae did not emerge during offshore winds, with associated low temperatures; instead, they responded faster than did controls to the appropriate cue provided by warmer

TABLE 5. Geometric mean ($\bar{X}$) values ± 2 SE for yolk length variables, and F-values from ANOVAs testing for differences among concentrations. $^{*}P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.0001$.

	Variable									
	OVERALL (μm)		MEAN (μm)		SLOPE (% reduction/d)		NIGHT/DAY (%)		END (μm)	
	$\bar{X}$	±2 SE	$\bar{X}$	±2 SE	$\bar{X}$	±2 SE	$\bar{X}$	±2 SE	$\bar{X}$	±2 SE
Nominal concentration (ppm)										
0	349	334–363	315	303–327	13.7	12.4–15.0	98	93–103	182	159–207
75	365	350–380	309	298–321	14.8	13.4–16.1	97	92–102	165	145–188
150	389	373–405	324	313–337	14.5	13.2–15.8	106	101–111	155	136–176
300	400	384–417	320	308–332	14.8	13.4–16.1	101	96–106	161	142–184
600	411	395–429	340	328–353	14.4	13.1–15.7	98	93–103	167	146–190
1200	412	396–430	345	332–358	14.0	12.6–15.3	104	99–109	176	155–201
F										
Concentration	10.9***		5.4**		0.4		1.9		0.8	
Oil versus control	30.6***		3.8		1.1		1.4		1.8	
Among oil	6.0**		5.8**		0.3		2.1		0.5	

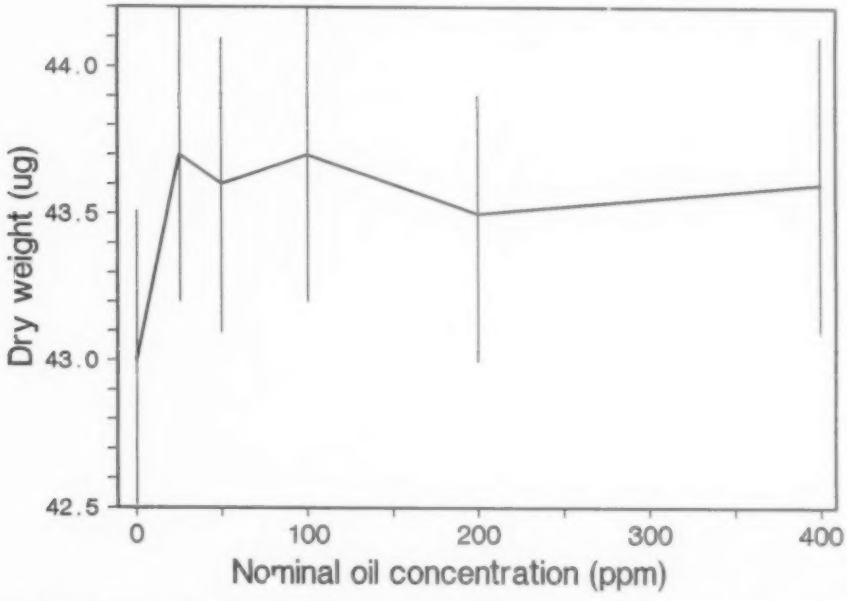

FIG. 8. Mean dry weights of emerged larvae. Values are means ± 2 SE, back-transformed from logarithms.

onshore winds. Accelerated emergence is a generalized response and is not associated with any specific oil compound or group of compounds. We used the same system to expose embryos to the WSF of Hibernia crude oil (unpubl. data). The WSF consists almost exclusively of mono- and diaromatics, yet induced the same accelerated emergence observed in the present study, in which aliphatics were the dominant compounds in the interstitial water.

Inhibition of growth and yolk utilization is also a generalized response and occurred when embryos were exposed to the WSF (Paine et al. 1988), as well as in this study. In this study, exposed larvae had yolks 10% longer, and heads 1% shorter, than control larvae emerging at the same time. Based on the observed 15% decline in yolk length per day, a 10% difference represents <1 d difference in developmental "age." Even this difference may be more apparent than real because of the differences in emergence patterns. Note that yolk length of exposed, unemerged larvae was not greater than that of controls. Perhaps exposure to oil increased the probability that larvae with large yolks would emerge, rather than actually affecting yolk utilization or developmental rate.

The 1% difference in body size (head length) between control and emerged larvae is unlikely to significantly affect survival. This difference is considerably less than the 10% difference we have observed for embryos exposed to the WSF (Paine et al. 1988), and the 100% difference observed for free-swimming larvae (i.e. no growth of exposed larvae) (unpubl. data). The reduced body size of larvae exposed to oiled sediments would also be compensated for by a greater yolk supply. In the absence

of direct toxic effects, exposure to oil can actually prolong time to starvation for capelin larvae by reducing the rate of yolk utilization (Paine et al. 1992).

It is difficult to estimate risks and critical concentrations from our experiment. Clearly, concentrations within the range tested would not produce significant negative effects, and accelerated emergence might be regarded as a positive effect. Higher concentrations could be tested, but these might decline more rapidly, if the sorptive capacity of the beach gravel is limited. Our other experiments with the WSF indicate the concentrations at which lethal effects occur, but the total hydrocarbon concentrations of the aromatic-rich WSF are not comparable with the total hydrocarbon concentrations of the aliphatic-rich interstitial water in the present study. The difference in composition plus the evidence that the effects observed in all our studies are generalized and not compound-specific also prevent any comparisons based on concentrations of individual compounds. The sum of C8–C26 *n*-alkanes would probably be the best measure to use if the dose–response relationships in this study were used to evaluate field samples collected after a spill.

Our experiments with the WSF indicated that hydrocarbon concentrations (total or individual compounds) producing lethal effects on capelin larvae are approximately an order of magnitude lower than those producing lethal effects on embryos (Paine et al. 1988, 1992). If the same were true for the hydrocarbons in interstitial water, then the greatest risk would occur if beaches were oiled just prior to, or coincident with, emergence. In the present study, larvae resided in the interstitial water for some time prior to emergence, but did not appear to be affected. However, hydrocarbon concentrations in the interstitial water had declined by up to 80–90% by that time. Lethal effects may also occur only when larvae are active and using yolk reserves. Activity of capelin larvae peaks at 8–9°C (Frank and Leggett 1982, 1983), which is also the temperature at which emergence peaks. As active larvae use the last remnants of their yolk, lipophilic hydrocarbons may enter the body (Solbakken et al. 1984). When capelin embryos were exposed just before hatch to concentrations of Hibernia crude oil WSF which were not directly lethal to the embryos, subsequent lethal effects on larvae occurred (Paine et al. 1992). In the present study, we were unable to examine delayed effects of embryonic exposure on larvae because the larvae were removed shortly after emergence.

The present study indicated that fish embryos could be successfully reared in sediment, and that tests of the effects of contaminated sediment in a flow-through system are certainly feasible. Emergence is also a sensitive response, although the response may not be negative. We recommend monitoring samples of emerged larvae in uncontaminated water, so that any delayed effects of embryonic exposure could be detected. Investigators have previously conducted experiments testing the effects of contaminants on salmonid emergence (e.g. Ostrander et al. 1988), although the contaminants are rarely added directly to sediments. The effects should be a function of contaminant concentrations in the interstitial water (e.g. Swartz et al. 1990), especially for nonfeeding embryos and larvae which cannot take up contaminants from food. For individual compounds, interstitial concentrations could be reproduced in solution, with no need to directly test sediment. However, for multiple contaminants such as the hydrocarbon mix in oil, it is simpler to contaminate the sediments rather than to prepare and test a solution reproducing the mix in the interstitial water.

Acknowledgements

Funding for this project was provided by the Office for Energy Research and Development. We thank Yannick Ducharme for her assistance throughout the study. Emilien Pelletier and Charles Brochu of INRS-Oceanologie conducted and helped interpret the chemical analyses. Mobil Oil provided the crude oil. Department of Fisheries and Oceans personnel from the Northwest Atlantic Fisheries Centre in St. John's, especially Jim Carscadden and Brian Nakashima, deserve thanks for providing logistical support. We also thank two anonymous reviewers and Beth Power for reviewing the manuscript. Finally, our sincere thanks to the people of Bryant's Cove for their assistance, interest, and hospitality.

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Modeling of Recruitment Patterns in Pacific Cod (*Gadus macrocephalus*) in Hecate Strait, British Columbia

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We combined published recruitment hypotheses into a single modeling framework and explored the relative contributions of the hypothesis factors to recruitment variation in Pacific cod (*Gadus macrocephalus*). Our five hypotheses were (1) recruitment decreased with increasing transport during the larval period; (2) recruitment was related to percentage hatching success as a dome-shaped curve with a peak between 4.0 and 5.0°C; (3) recruitment was positively related to abundance of age-0 juvenile Pacific herring (*Clupea harengus pallasi*) hatched in the same year as the cod; (4) recruitment was positively related to abundance of age-0 and age-1 herring in the year prior to spawning; and (5) a dome-shaped curve related recruitment to adult stock size. We used new records for temperature, and a new index of advection based on measures of current in Hecate Strait. For fitting a response surface with age-3 cod recruitment as the independent variable, we used a 20-yr data series of variables from 1962 to 1981 inclusive and reserved data for the years 1982–85 for a prediction test. Only hypotheses 1 and 5 were supported by both the response surface fit and prediction tests. The stock–recruitment curve can be fitted only when the transport curve is fitted simultaneously.

Les auteurs ont regroupé cinq hypothèses publiées sur le recrutement en une seule structure de modélisation et ont étudié hypothèse à la variation du recrutement de la morue du Pacifique (*Gadus macrocephalus*) dans le détroit d'Hécate. Ces cinq hypothèses sont 1) le recrutement diminue en fonction de l'augmentation du transport pendant la période larvaire; 2) le recrutement est lié au pourcentage du nombre d'oeufs éclos et a l'allure d'une courbe en dôme présentant un pic entre 4,0 et 5,0°C; 3) le recrutement présente une relation positive avec l'abondance de harengs du Pacifique (*Clupea harengus pallasi*) juvéniles d'âge 0 nés la même année que la morue; 4) le recrutement présente une relation positive avec l'abondance des harengs d'âges 0 et 1 au cours de l'année précédant la fraie; et 5) une courbe en dôme décrit la relation entre le recrutement et la taille du stock d'adultes. Les auteurs présentent de nouvelles données sur la température et un nouvel indice de convection horizontale basé sur des quantifications du courant dans le détroit d'Hécate. Pour le lissage de la surface de réponse basé sur le recrutement de morues d'âge 3 comme variable indépendante, ils ont utilisé une série de données couvrant 20 ans, soit de 1962 à 1981 inclusivement, tout en réservant les données recueillies de 1982 à 1985 pour la réalisation d'un essai provisoire. Seules les hypothèses 1 et 5 ont été étayées par le lissage de la surface de réponse et l'essai provisoire. La courbe de recrutement du stock ne peut être lissée que lorsque la courbe du transport est lissée en même temps.

Received August 20, 1990
Accepted May 2, 1991
(JA693)

Reçu le 20 août 1990
Accepté le 2 mai 1991

The stock of Pacific cod (*Gadus macrocephalus*) in Hecate Strait has been studied for over 30 yr by biologists at the Pacific Biological Station, and as a result, reliable time series indexing change in total stock size and recruitment for the period 1962 to present have been developed. The stock is well defined biologically, spawns within an area of 50 by 130 nautical miles (Hecate Strait), and is fished consistently in a classical target-species fishery by a small fleet of medium-sized trawlers with known characteristics (Westrheim and Foucher 1985).

The species is ideally suited to a study of recruitment dynamics. A high annual natural mortality rate of 45% combined with an equally high fishing mortality rate causes the population to replace itself every few years (Tyler and Foucher 1989). Fish older than age 7 are rare in the fishery. Individuals grow rapidly, most reaching 43–48 cm after 2 yr (Tyler and Foucher 1990). Recruitment variability at ages 2 and 3 is easy to observe, with large year-classes quickly detectable in length–frequency samples of the catch by the fishery. The dynamics of recruitment essentially controls fishery production with little lag. The stock has had three major decreases in abundance followed by increases (Tyler and Westrheim 1986). The reversal of stock trends and the strong recruitment changes make clear signals for investigation.

In an earlier study, Walters et al. (1986) explored a predator–prey relationship between cod and Pacific herring (*Clupea harengus pallasi*) using linearizations of the Ricker stock–recruitment function with predation terms added. They found that historic fluctuations of both fish species could be largely

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accounted for by this predation hypothesis. Commercial fishermen agreed that the recruitment trends were real and independently proposed that cod year-class strength was related to the abundance of forage fish including herring and Pacific sand lance (*Ammodytes hexapterus*). In a second publication, Tyler and Westrheim (1986) set up alternate hypotheses concerning physical oceanographic factors and used the Schnute–McKinnell analysis of curvilinear response surfaces (Schnute and McKinnell 1984). This statistical work indicated that northward advection while the fish were in larval stages (January through March) could explain most of the variance. Ambient temperature during egg hatching and larval development did not account for variance as well as did transport. While the modeling approaches of the two research teams did not allow direct statistical comparisons of the two sets of hypotheses, it appeared that both physical factor influences and prey availability were tenable in a preliminary sense as alternative explanations, since both could explain the major trends in recruitment of Pacific cod.

The advection index used earlier (Tyler and Westrheim 1986) was from Ekman transport calculations (Bakun 1973) and was questionable for our application because it was based on winds calculated at an offshore point some distance from Hecate Strait. To investigate the transport hypothesis further, we decided to develop for this paper a more direct index of northward advection by comparing water transport calculations from direct current measures in 1983 and 1984 with a number of surrogate variables in Hecate Strait (Crawford et al. 1988).

An especially strong year-class of Pacific cod occurred in 1985 (Tyler and Foucher 1989). Year-classes of similar strength occurred previously in our time series in only two intervals: each year during the run of years 1962, 1963, and 1964 and again in 1970. We considered that any useful model would have to be capable of predicting an event such as occurred in 1985. Our reasoning is that when fluctuations are near the mean of a series, say within 10%, the mean itself is in some sense a good predictor. But when a strong positive or negative deviation occurs after a period of minor fluctuations, prediction becomes more problematic and is more influential in subsequent stock size change. We therefore adopted prediction of the 1985 year-class as a test of our model. In the earlier herring–cod modeling by Walters et al. (1986), no attempt was made to predict forward in time, since the objective was limited to gaining insight in processes. Tyler and Westrheim (1986) attempted to predict the 1985 year-class using a model incorporating sea-surface temperature in February, mean transport in January, February, and March, and parent stock size. The attempt failed in that the predicted value for the year-class strength turned out to be only 28% of the value ultimately observed.

The objective for the present study was the reexamination of historic recruitment time series using the new advection measure simultaneously with the herring predator–prey hypothesis, all taken within a single framework so that the relative explanatory power of each could be explored. We used the Schnute–McKinnell multivariate analysis with second-order equation fitting to achieve this because it was suitable for exploring the curve shapes generated by all hypotheses in a simple curvilinear response surface.

Methods

Development of Hypotheses and Data Series

To reduce the possibility of spurious correlations, we developed a priori hypotheses by a procedure that we have come to call the "Events Method." By this procedure we laid out what is known about the life history of the Pacific cod, especially in Hecate Strait, and determined the space occupied by a cohort in its various life history stages. We then listed the known events that might have been influential in the survival of the cohort in each stage and place, up to the point when its relative strength became evident in the fishery (age 3). In a workshop designed to bring together expertise from areas of ecology and physical oceanography, we outlined the possible relationships between cod and other species and the physical oceanographic phenomena (Tyler et al. 1986). Oceanographers familiar with the region noted that northward transport was a major feature in the Strait during late winter when the cod were in larval stages.

Transport Hypothesis

The first hypothesis, advection during the larval period, accounted for more of the interannual variance than any other physical variable in the earlier study (Tyler and Westrheim 1986). The hypothesis was that recruitment decreased because the young were lost to the population. We did not specify the cause of mortality. The phenomenon that first attracted our attention was that northward winds are often strong and persistent between November and April (Dodimead 1980) whereas they are generally weaker and southward during the summer months. The winter winds bring about a high degree of turbulence, mixing waters to depths as great as 100 m (Dodimead 1980), whereas in the summer a pronounced pycnocline forms. Nontidal current in winter has been measured as high as 10 cm/s with oceanographic moorings in the southern portion of the Strait and 50 cm/s in the shallower, more restricted northern portion (Crawford et al. 1988).

Current-meter data from the Strait were used to derive the new surrogate measure of current that could be used through the entire period under analysis (Crawford et al. 1988). Current meters were deployed between May 1983 and April 1984 along three lines across Hecate Strait. Water transport through the Strait was calculated from these measurements. The fluctuations in winter transport (January through March period as in Tyler and Westrheim 1986) were correlated with a number of surrogate indicators. All time series were passed through a 2-d low-pass numerical filter to eliminate the influence of tidal currents on the correlation, since the oscillating motion of the tide carried no net transport through the Strait. The best correlation ($r = 0.91$) was with the cross-strait difference in subsurface pressure measured with moored meters midway along the Strait, but this series is available for the period May 1983 to April 1984 only (Crawford et al. 1988). We could use a single winter of data to investigate interannual variations for the following reasons. Storm winds drive both the average and fluctuating transport in winter through the Strait (Crawford et al. 1988). The winter flow northward is generated by individual storms, and the winter average of the cross-strait pressure gradient, and the sea level rise at Prince Rupert will be the average of those due to individual storms. Therefore, we expect the dynamics and statistics of the within-year fluctuations to be reasonable indicators of the interannual variations.

Among the surrogate series available for 1962–85, the best correlation ($r = 0.85$) with transport for the winter of 1984 was the subsurface pressure at Prince Rupert at the northeastern corner of the Strait. This series was formed by adding air pressure in millibars, measured at Prince Rupert Airport (January

through March), to sea level in centimetres, measured at Prince Rupert Harbour. One centimetre of seawater exerts a pressure of 1 millibar, within 1%. Subsurface pressure was chosen for the correlation to eliminate the influence of air pressure on sea level. This correlation is high because the northward winds in winter, driving the transport, pile up the water at the northern, constricted end near Prince Rupert. Geostrophic adjustment of the sea surface due to northward currents through the Strait also raises sea level along the eastern shore, contributing to the correlation (Crawford et al. 1988).

The linear fit between calculated transport and Prince Rupert subsurface pressure is given by $T = -54.67 + 0.39P$ where T is transport expressed as 10^6 m^3/s and P is subsurface pressure measured in kilopascals (1 millibar = 0.1 kPa). Averge winter transport from January through March was 0.33×10^6 m^3/s with a standard deviation of 0.31×10^6 m^3/s. Subsurface pressure measured at the depth of chart datum in Prince Rupert Harbour was 141 kPa for the same period.

Temperature Hypothesis

The second hypothesis that we investigated was that recruitment was related to ambient temperature through success at hatching as a dome-shaped curve with a peak between 4.0 and 5.0°C. We chose these temperatures because a published response surface analysis of percent hatch success as a function of temperature, salinity, and oxygen concentration indicated that peak survival occurs at 4–5°C for full oxygen levels and salinities of 25–30 ppt as occur in the Strait (Alderdice and Forrester 1971; Schnute and Mckinnell 1984). As a temperature record in our earlier physical factor model for cod recruitment, we used the McInnes Island lighthouse data for sea-surface temperature (SST) because it was taken through a period that included the entire biological time series that we wished to analyze. However, we decided in the present analysis to use the SST series taken at Bonilla lighthouse in the middle of the Strait (north to south) that starts in 1960 and that was closer to the spawning area of the cod (Foucher et al. 1989; Westrheim 1977). We consider that SST is useful as an index of ambient temperature for spawning cod because the water column is mixed to a depth of at least 100 m in late winter when spawning occurs. The coldest mean monthly temperatures during February recorded for the series that we used in the present analysis (1962–81) was 4.4°C in February 1969 and 4.4°C again in February 1972, while the warmest temperatures were 7.6°C in both February 1963 and February 1977 and 7.7°C in February 1981. We would expect from the published laboratory work that recruitment would be inversely related to temperature over the observed range of 4.4–7.8°C (mean = 6.2°C), since the hypothetical optimum (4.0–5.0) is at the low end of that range.

Herring as Prey for Juvenile Cod

The third hypothesis that we developed was related to the effect of 0-group herring as prey for 0-group cod, specifically that recruitment was positively related to the abundance of juvenile herring hatched in the same year as the cod brood. We refer to this variable as HERR 0. To puruse this analysis within the present modeling context, we used the abundance estimates of 0-group herring extrapolated from age-3 and older abundances calculated from Fournier stock–reconstruction procedures with commercial fishery data (Haist et al. 1988). We summed the year-class strengths from three stocks that are present in the Strait: "Northern," "Central," and "Queen Charlotte." This was exactly the treatment of data by the earlier study (Walters et al. 1986).

Herring as Prey for Adult Cod

Our fourth hypothesis was that recruitment was positively related to the summed abundance of age-0 and age-1 herring 1 yr prior to cod spawning (HERR 0,1), which assumes that when prespawning cod feed well they produce a stronger year-class due to greater fecundity or quality of eggs. We incorporate a minor change of data use from the earlier work (Walters et al. 1986) in that they summed ages 0, 1, and 2. Ages 0 and 1 were used here because they are in the same length range as sand lance when they occur in Pacific cod stomachs (1045 sand lance were measured from cod stomachs; Westrheim et al. 1989). A few herring that were possibly age 2, ranging 16 to 20 cm, were found in stomachs, but the sample size was small (18 of a total of 58 herring found in cod stomachs; Westrheim et al. 1989).

Recruitment and Adult Stock Size Hypothesis

Our final hypothesis was that recruitment was related to parent stock size through a dome-shaped curve. Previous work with this stock indicated that it was possible to represent adult cod abundance changes with a catch-per-unit-effort (CPUE, kilograms per hour) series standardized by vessel horsepower and refined by using only those commercial trips that targeted on Pacific cod and had 20% or more of a haul of this species (Westrheim and Foucher 1985, 1986). Because the effort data were not comprehensive prior to 1962 we started our CPUE series in that year. The catches were nominal in that they were not corrected by adding discards at sea due to "high grading." The proportion discarded would be greatest in years when particularly strong year-classes recruited, thus leading to underestimates of these cohorts. The stock size index also has some measurement error because only a small portion of the age-2 fish are mature, and about 70% of the age-3 fish are mature. Nearly all of the age-4 fish are mature (Westrheim 1977). It would be better to convert biomass into egg production, but fecundity estimates are not yet available for the stock, although work on that aspect is in progress.

Recruitment Variable

Age determination was carried out by length–modal analysis (Foucher and Fournier 1982). To estimate the interannual fluctuation of recruitment, we had a choice of using published virtual population analysis (VPA) or catch-per-unit-effort at age 3 (CPUE-3), the first age of full recruitment. Length–modal analysis tends to produce greater percentage error for the older ages (Beamish et al. 1990). Since VPA is a back-calculating procedure for catch-at-age that uses all ages, it would incorporate the error of the older ages. To obviate this effect of aging error, we used CPUE-3, since only the aging error associated with age-3 fish would be incorporated. Thus we expressed recruitment in the analysis as the previously published catch rate in numbers of age-3 fish per hour of trawling, targeted on cod (CPUE-3), and standardized for horsepower. The length/age sampling procedures that we used, the method for breakdown of the catch by age groups, and the procedure to standardize catch rate by horsepower were developed by Westrheim and Foucher (1985).

The 1985 year-class was one of the strongest for the entire data series. Consequently we considered it would be a partic-

ularly useful test if our model could predict this phenomenon. We used data from 1962 to 1981 inclusive for fitting our model, and then used the more recent data from 1982 through 1985 to determine how well the model predicted these year-classes.

Statistical Fitting Techniques

Initially we looked at scatter plots of the independent variables against one another and the correlation coefficients (r) among them to check for independence. We next examined the dependent variable (CPUE-3) against the five independent variables in the same manner.

Our main analytical approach was to use a multivariate, second-order, curvilinear fitting technique (Schnute and McKinnell 1984) to examine a series of fittings (models) for various combinations of the five independent variables, including a series of models with one, two, and three independent variables. The multivariate approach was used to analyze two and three independent factors simultaneously. Second-order curve fitting was deemed to be appropriate for the hypotheses being investigated. The curves that can be generated by the method included skewed and symmetric parabolas that may be parameterized as nearly a straight line or as steeply curved and either open upward or downward. The range of possibilities covers the dome-shaped curves considered to be likely for the recruit–temperature and recruit–stock relationships, as well as nearly linear relationships with transport, or asymptotic curves or power curves that might describe relationships with forage fish. The objective function was the likelihood statistic. The equations used in the Schnute–Mckinnell procedure are summarized in the Appendix.

We used the likelihood statistic to rank the relative fit of the response surface between the CPUE-3 data and the independent variables. In addition, we calculated the r^2 values between the observed and fitted CPUE-3 values of each model and ranked the models of the same dimensionality. Factors that were ranked first or second in each case were considered the most likely models to "explain" the response variability. Schnute and McKinnell's "B statistic" was designed to test whether the variance reductions from models of increasing dimensionality were significant, but the method could not be used in the present application because (1) repeated testing over a series of models invalidates interpretation of the F-table probability levels in the sense that if a test is repeated often enough the null hypothesis will eventually be rejected and (2) we used time series data in which adjacent year population responses are only pseudo-replicates of one another, so degrees of freedom are lower than the number of time intervals, and could not be calculated precisely.

Results

Scatter Plots and Linear Fitting

To test whether any of the independent variables were significantly correlated with each other, we calculated the linear regression coefficients of each of these variables with all the others. The multivariate model would not be statistically valid if independent variables were correlated. Only two pairs of variables had r values that were significant at the 5% level. Transport was significantly correlated with temperature ($r = 0.47$, df = 18; Fig. 1A), and the adult cod stock size was significantly and inversely correlated with the summed number of juvenile herring of ages 0 and 1 in the year prior to cod spawning (HERR 0,1) (Fig. 1B). These pairs of variables were subsequently not used in the same multivariate model. A time plot shows an alternation between adult cod and juvenile herring that could be interpreted as a predator–prey relationship (Fig. 2). We emphasize that the two herring variables

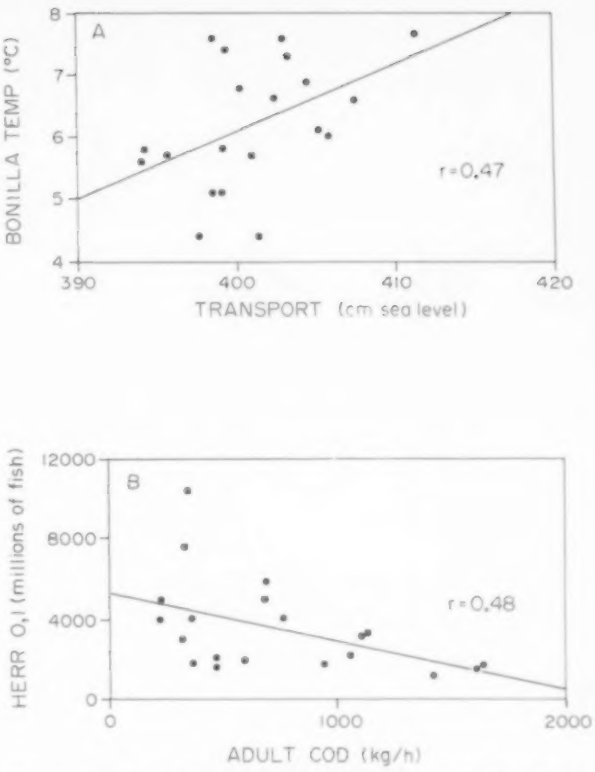

FIG. 1. Statistically significant correlations ($p = 0.05$) between "independent" variables. (A) Bonilla Island lighthouse sea-surface temperature (SST) versus the transport index (TRANS, January, February, and March, at the Prince Rupert hydrographic station adjusted for atmospheric pressure); (B) herring juveniles summed for ages 1 and 2, hypothetically as forage for adult cod the summer before cod spawning occurs (HERR 0,1), versus CPUE for adult cod (ADULTS).

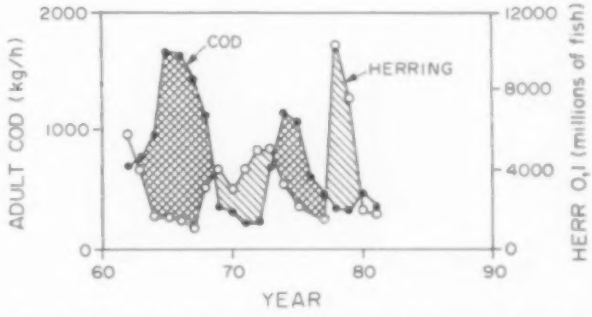

FIG. 2. Time series of herring juveniles summed for at ages 0 and 1, hypothetically forage for cod the summer before cod spawning occurs (HERR 0,1) plotted along with CPUE for adult cod (ADULTS) showing the inverse alternation that could be due to a mutually controlling predator–prey relationship.

(HERR 0,1 and HERR 0) were not significantly correlated and had an r value of only 0.19.

We next examined the linear regressions of the response variable (the cod recruitment index, CPUE-3). No relationship was evident when CPUE-3 was plotted against each of the herring variables (HERR 0 and HERR 0,1, $r = 0.06$ and 0.13, respectively). The mean number of 0-group herring for the series was 2078×10^9 fish. For the other herring variable (HERR 0,1) the mean number of juveniles in a year was 3526×10^9 fish (Fig. 3A and 3B).

The relationship between cod recruitment and transport was significant ($r = 0.43$, $p < 0.05$). Only low recruitment occurred at transport index values greater than 404.5 cm (Fig. 3C). The very strong year-class of 1962 coincided with the lowest transport index value of the series (394.2 cm), although both low and high recruitment occurred at low transport values, possibly indicating the influence of other factors acting simultaneously. The mean transport index value was 401.1 and the range 394–411 cm.

Although SST and transport were themselves correlated, there was no significant correlation between CPUE-3 and SST ($r = 0.15$). The mean February temperature for the period of analysis was 6.2°C with a range of 4.4–7.7°C. The highest cod recruitment occurred in 1962 at a mean SST of 5.8°C (Fig. 3D). High and low year-class strengths occurred at the high temperatures of 7.3–7.7°C. In 1963, 1964, and 1970, both temperature and recruitment were high. In 1977 and 1981, temperature was high but recruitment was low.

The relationship between recruitment and parent stock size had no significant linear correlation ($r = 0.21$). There was, however, a visual hint that a second-order dome-shaped curve may apply (Fig. 3E), since there appeared to be a "hollow" in the scatter plot for the ADULTS index values of 689–1064 kg/h. All CPUE-3 recruitment levels for that range of ADULTS were above the mean CPUE-3 index value of 102. The high recruitment levels were not only mostly towards the center of the stock size range, but they were also from years when transport was less than average as can be seen by comparing Fig. 3C and 3E. The time course of stock size and recruitment changes (Fig. 3E, arrows) started in the near center of the range of the parent stock size in 1962. Recruitment decreased as stock size built up until 1966. The persistent decline in recruitment brought about a decrease in the stock size. Increases in recruitment subsequently occurred as the parent stock thinned in the years 1969–73, so in consequence, by 1973 the stock size was increasing again and continued to increase in 1974. Poor recruitment subsequent to 1974 lead to a decrease in stock size in 1976 and 1977. The stock size remained at this small size through the rest of the time series because recruitment rates were low. These dynamics suggested to us a relationship between stock and recruitment, in that the sequence of change was for high stock size to be followed by low recruitment and the resulting low stock size to be followed by high recruitment. That the track did not retrace itself on the graph may be due to either an influence by other factors boosting recruitment in the early 1960's and lowering it in the early 1980's or to the measurement error described in the Methods section in the recruitment and stock size variables.

Multivariate Fitting

We fitted a series of eight, two-factor models using the valid combinations of pairs of independent variables. The models

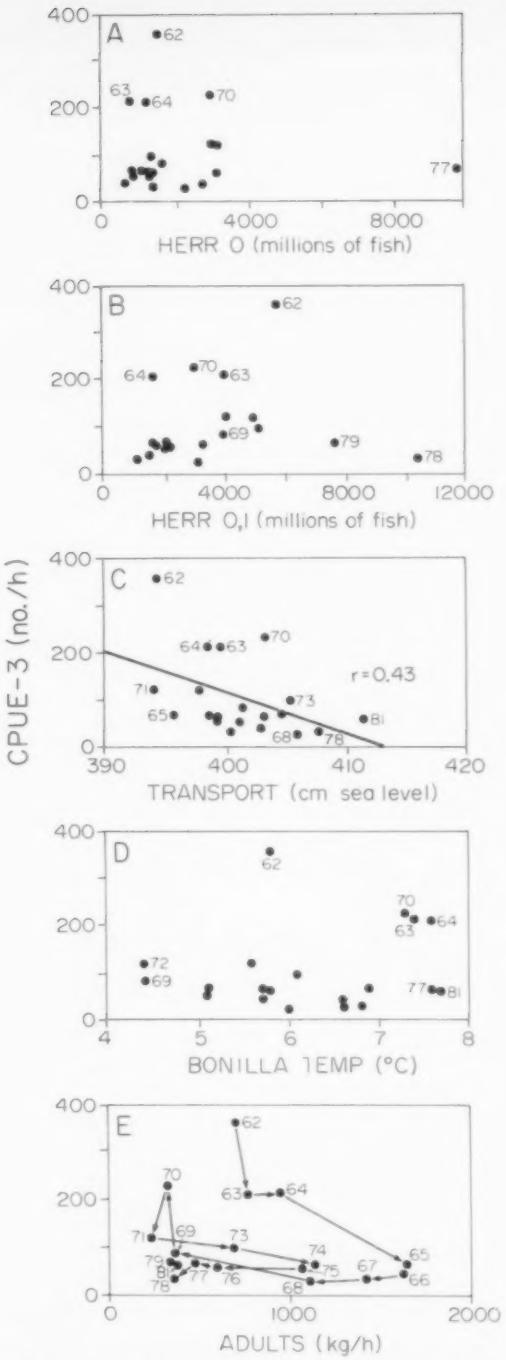

FIG. 3. Scatter plots between the dependent variable of year-class strength (CPUE age-3 cod (CPUE-3)) versus (A) young of the year herring hatched in the same year as the cod (HERR 0), (B) juvenile herring summed for ages 0 and 1, hypothetically forage for adult cod the summer before cod spawning occurs (HERR 0,1) (C) mean northward transport index in the first quarter of each year (TRANS, at the Prince Rupert hydrographic station adjusted for atmospheric pressure), (D) Bonilla Island lighthouse sea-surface temperature for February (SST), and (E) CPUE for adult cod (ADULTS). Arrows in Fig. 3E indicate the transition among years.

TABLE 1. Relative, ranked goodness of fit of two- and three-factor models. The likelihood statistic measures the goodness of fit to the response surface, the lowest values having the least variance. The r^2 statistic measures the percent of the observed recruitment values "explained" by the fitted values. Variables are as follows: SST, sea-surface temperature (°C); TRANS, transport index (cm sea level); HERR 0,1, abundnce of juvenile herring ages 0 and 1 (millions of fish); HERR 0, abundance of age-0 herring (millions of fish); ADULTS, abundance of mature cod (kg/h catch rate).

Factors	Likelihood statistic ($\times 10^{-3}$)	r^2 (%)
Two-factor models		
SST/HERR 0,1	95	50
TRANS/ADULTS	107	60
ADULTS/HERR 0	124	28
TRANS/HERR 0,1	127	38
HERR 0,1/HERR 0	144	26
TRANS/HERR 0	155	33
SST/HERR 0	169	14
SST/ADULTS	172	12
Three-factor models		
SST/HERR 0,1/HERR 0	48	78
TRANS/ADULTS/HERR 0	61	72
SST/ADULTS/HERR 0	65	55

were represented as response surfaces with CPUE-3 as the dependent variable. We ranked them by goodness of fit by the likelihood statistic (smaller values indicated reduced variance around the response surface; Table 1). We also calculated the r^2 values in percent for the correlation between the observed and the fitted points to express the fit as percentage of variance explained (Table 1). Two models stand out: the SST/HERR 0,1 model accounted for 50% of the variance (r^2) and the TRANS/ADULTS model had an r^2 explaining 60%. The r^2 values of the other models ranged from 14 to 38%. Since the next best model (ADULTS/HERR 0) was much worse in relative fit, we consider that only the factors of the two best models were supported by these procedures: SST, TRANS, ADULTS, and HERR 0,1. In fact, adding the variable HERR 0 to TRANS, the latter significantly correlated with CPUE-3 on its own, did not produce the good fit that adding ADULTS to TRANS did. These differences in relative fit seem to indicate that the hypothesis involving HERR 0 is not supported.

We next examined the relative goodness of fit of each of the four valid three-factor models. The fit for TRANS/HERR 0,1/HERR 0 did not converge on a single set of parameters in the iterative fitting procedure. Because the model could not be fitted statistically and parameters could not be calculated, this model had to be discarded. Adding HERR 0 to the two factor model with SST/HERR 0,1 turned the best two-factor model into the best of the three-factor models (Table 1, $r^2 = 77.9\%$). Adding HERR 0 to the model TRANS/ADULTS produced a fit that was nearly as good, however ($r^2 = 72.0\%$). Adding HERR 0 to the SST/ADULTS model helped what was the poorest of two-factor models, but the r^2 value of 55% was not nearly as good as the other three-factor models. Because the two best of the three-factor models contain all five of the factor hypotheses, the three-factor models seem not to distinguish whether any of the hypotheses should be discarded.

Prediction Test

Exploration of predictability was the next step in testing the models. The 1985 year-class was over three times as large as

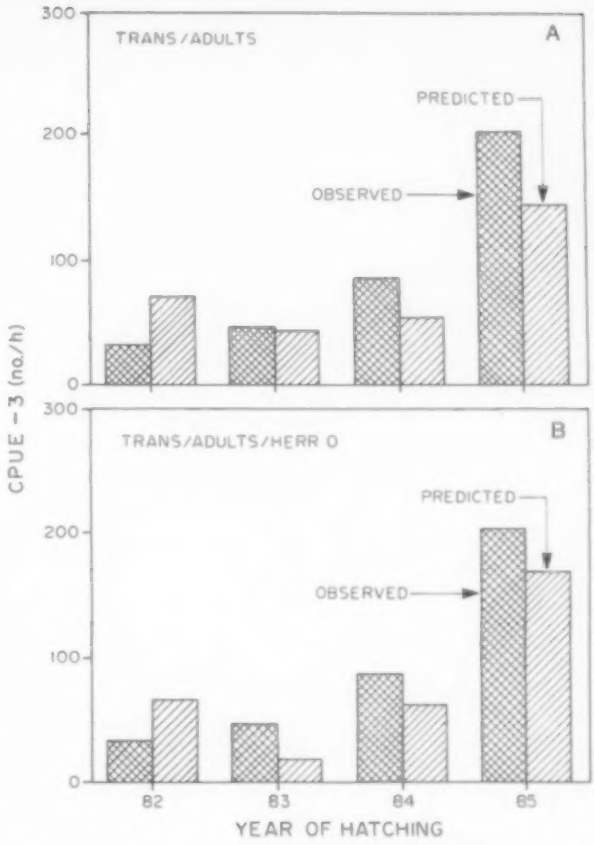

FIG. 4. Observed and predicted year-class strength (CPUE of age-3 cod (CPUE-3)) during the 4 yr of the time series since the fitted data series ended for the best (A) two-factor and (B) three-factor models.

any year-class between 1978 and 1983 and about twice the size of the 1984 year-class. In addition to examining the 1985 prediction, we calculated the mean absolute difference (d) between the observed (o) and predicted (p) values as a percentage of the observed, termed below the "mean percentage difference" [$d = 100 \times \text{ABS}(o - p)/o$]. The best two-factor model, SST/HERR 0,1, missed the 1985 year-class (predicted only 57 fish/h whereas the observed value was 203 fish/h). The year-class strength for 1983 was wrongly predicted as well, calculated as 5.0 times larger than the observed (233 versus 47/h, respectively). The mean percentage difference was large: $d = 165\%$. Because of this poor performance, we discarded the SST/HERR 0,1 model.

The second-best two-factor model, TRANS/ADULTS, gave a prediction for 1985 that was fairly close: 73% of the observed value (148 versus 203 fish/h, calculated versus observed; Fig. 4). The mean percentage difference, $d = 49\%$, was much better than for the SST/HERR 0,1 model. Because predictions for all years were reasonably close, we decided to consider this model further. We next used the model to make predictions for more recent years for which the observed data were not yet compiled at the time of writing. These values for CPUE-3 were 50 fish/h in 1986, 51 fish/h in 1987, 45 fish/h in 1988, and 162 fish/h in 1989. We noted that recruitment for 1986–88 was substantially below the 20-yr mean of 102 fish/h for the fitted

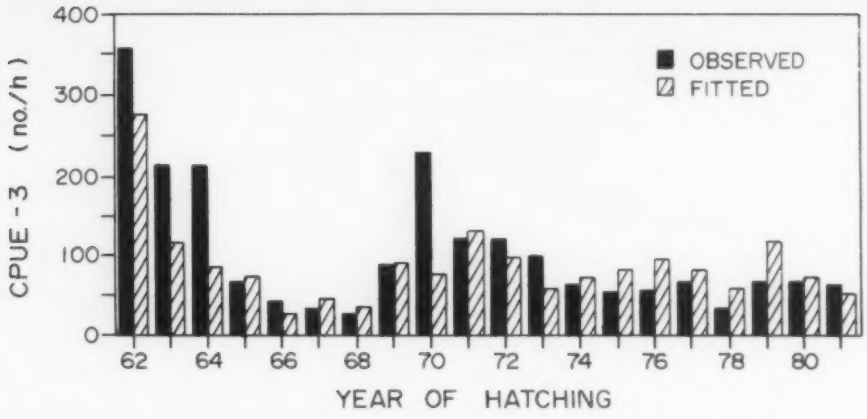

FIG. 5. Time series of observed versus fitted year-class strength (CPUE of age-3 cod (CPUE-3)) using the two-factor model with independent variables for northward transport and adult biomass.

period 1962–81, while recruitment for 1989 was predicted as above the mean. As fishery data are compiled over the next few years, these predictions could be tested. The fitted parameters for the model are given in the Appendix. The time plots of the observed and fitted data showed that the main trends in recruitment were captured by the fit but that the highest observed peaks were underestimated (Fig. 5).

For the three-factor models, only one predicted the 1985 year-class, TRANS/ADULTS/HERR 0. Also, the mean percentage difference for the TRANS/ADULTS/HERR 0 model was d = 55% compared, respectively, with 175 and 491% for the other three-factor models: SST/ADULTS/HERR 0 and SST/HERR 0,1/HERR 0. We concluded that TRANS/ADULTS/HERR 0 was the best three-factor model but that there was little improvement to the fit between observed and predicted values gained by adding the third factor, HERR 0 (Fig. 4). The variables TRANS, ADULTS, and HERR 0 could not be rejected by the prediction test, although TRANS and ADULTS were sufficient for modelling the data in hand. It appears that the three-factor models are not useful.

Response Surface

The relationship between recruitment, adult stock size (ADULTS), and transport (TRANS) may be viewed as a series of curves of equal-value isopleths of the CPUE-3 variable expressed in numbers of fish per hour (Fig. 6). The highest isopleth is to the left in the figure at 125 fish/h, and the lowest is 25 fish/h, the isopleth series decreasing in intervals of 25/h. For any one level of stock size, recruitment decreases with increasing transport. For any one level of transport, recruitment increases as stock size decreases down to the ADULTS values of 400–500 kg/h, after which recruitment decreases. The levels 400–500 kg/h represent a critical stock size below which recruitment falls off.

In addition to the recruitment contours, we also plotted the positions of the data points on the surface and the residuals (Fig. 6). The points were well distributed over the area, and only four residuals stood out with data points being well above the fitted surface. These were the four highest recruitment values, occurring in 1962, 1963, 1964, and 1970 (see also Fig. 3). Either an additional factor was necessary to account for these

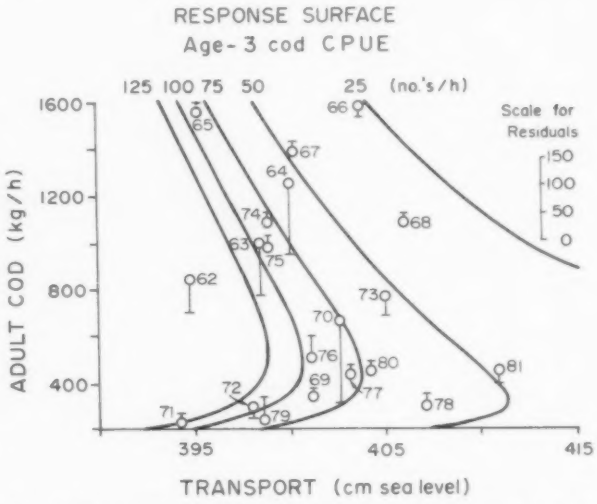

FIG. 6. Topographic display of the fitted response surface of the two-factor model with independent variables for northward transport (TRANS) and adult biomass (ADULTS) and the response variable of year-class strength expressed as standardized CPUE of age-3 fish (CPUE-3). The residuals between the observed year-class strength (circles) and the fitted surface position are given as vertical lines. The short horizontal lines represent the position of the response surface corresponding to the year-class strength point.

year-classes or we did not measure the magnitude of extreme year-classes well.

For clarity, we examined the shape of the response surface by computing slices through the surface at selected values of the independent variables. When we made a cut at the critical stock size of 500 kg/h through the range of transport values, the resulting relationship between recruitment and transport resembled a negative power curve, with recruitment falling with increased transport (Fig. 7A). When the slice was made at the mean value for transport and through the range of adult stock sizes, the curve was reminiscent of a Ricker stock–recruitment curve (Ricker 1975) and the critical stock size was quite clear (Fig. 7B).

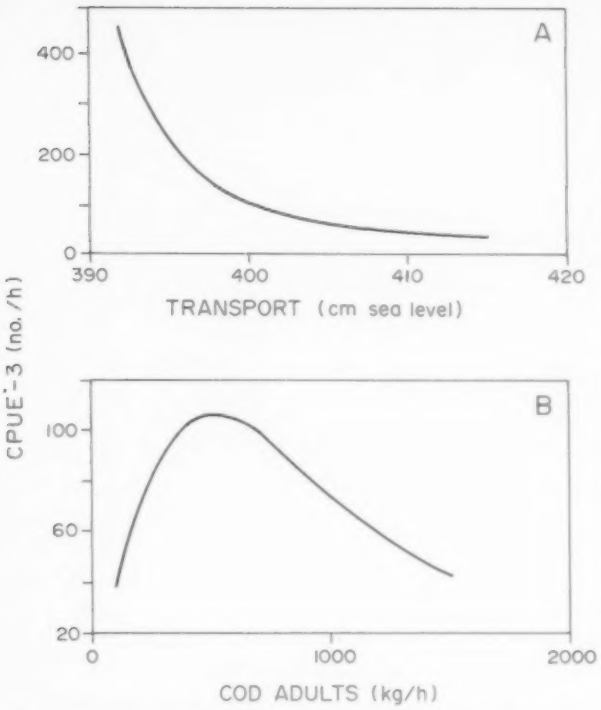

FIG. 7. Cuts through the fitted response surface (of Fig. 6) of the two-factor model with independent variables of northward transport (TRANS) and adult biomass (ADULTS) and response variable of year-class strength expressed as standardized CPUE of age-3 fish (CPUE-3). (A) fitted relationship between year-class strength and transport at the stock size for maximum recruitment; (B) fitted relationship between year-class strength and the adult stock size index at the mean transport value.

Discussion

We recognize the limitations of the correlative approach in the investigation of cause and effect relationships and have attempted to develop a means to avoid the inclusion of spurious relationships (Beverton 1989; Botsford et al. 1989). In our earlier studies, we used correlations simply as an exploratory method of developing models of alternative factors that could plausibly cause changes in recruitment rate. Our position was that the historic data series had information content that would help with hypothesis development. The stock showed strong fluctuations, both increasing and decreasing trends, as well as strong recruitment events that were likely not to be obscured by or due mostly to measurement error. Spurious correlations would be more likely if the recruitment and stock size trends are simple, say, a simple decreasing trend or a decreasing trend followed by an increase. Because the recruited stock showed two increasing periods and three decreasing periods over the 23-yr interval between 1962 and 1985, spurious correlations are made less likely by the nature of the response variable itself.

In the present study, we went further in trying to sort out spurious correlations. First, we constructed a new time series of water-mass transport and also switched to a temperature series that was more relevant to the temperature–recruitment hypothesis. We also reformulated one of the herring variables (HERR 0,1). While we found that these new independent variables were all correlated with the original variables, the differences in the variance structure could have substantially weakened the relationships with recruitment. In the case of transport, however, the linear correlation with recruitment was strengthened. Second, we went further and tested whether the fitted models would predict a major recruitment event not incorporated in the fitted portion of the series and found that the models could predict it.

There are other testing procedures that we can use in the future to evaluate our model. We have the opportunity to examine the same or similar hypotheses on another stock of Pacific cod off southwest Vancouver Island (La Perouse Bank). We know that the stock trends for Hecate Strait and La Perouse Bank are not correlated, and so we would be working with independent recruitment events. Another possibility is that we may be able to get the cooperation of the trawling industry to conduct a fishing experiment that would uncouple "normal" event sequences and, as suggested in Walters et al. (1986), allow us to probe the apparent density dependence that we have found in the Hecate Strait stock. We could, for example, foster high fishing effort on cod when cod are low and herring are high in abundance to see whether herring would stay high in the comparative absence of cod. Finally, we have begun to examine changes in growth, fecundity, egg stage and size, and age and size at first spawning in relation to stock size changes and will be able to refine the spawning adult variable and find other evidence of density dependence.

It is a general limitation of ecological studies of large populations that insight into causal relationships must be built slowly through repeated observations of sequential events and by observations cross-checked with logical connections, for example, stomach content studies that might or might not be supportive of the time series models. If enough cross-checks are made so that it is difficult to account for the patterns except by the proposed model, then the model becomes useful, and especially so when it has predictive power. A central question to this paper is whether we can accept the observations presented here as evidence for cause and effect relations. Although we have just proposed that additional testing of the model is feasible, any one test will not likely in itself be really convincing. Although in the strictest sense nothing can be proven, repeated success at prediction is useful and gives insight. The present publication represents the third study of factors that possibly influence the recruitment patterns of the Hecate Strait stock of Pacific cod. We believe that the work is now past the exploratory hypothesis stage and that the evidence is beginning to accumulate that supports causality. The present study found support for density dependence in recruitment and influence of transport.

Our study found that cod recruitment patterns were not as tightly related to the abundance of juvenile herring as to transport and adult stock size. Still the fact that stock size and density dependence were supported indicated that there likely was food limitation related to compensatory mortality at one or more life history stages. Although not dealt with in this paper, density dependence at larval stages was not to be ruled out here, while the significant inverse correlation between adult cod abundance and juvenile herring (Fig. 1 and 2) pointed to the possible influence of the abundance of age-1 and age-2 herring as forage. Also, there are published stomach content analyses (Westrheim 1977) verifying that herring is a major food item of Pacific cod (Fig. 8). The low ranking of the herring variables as factors

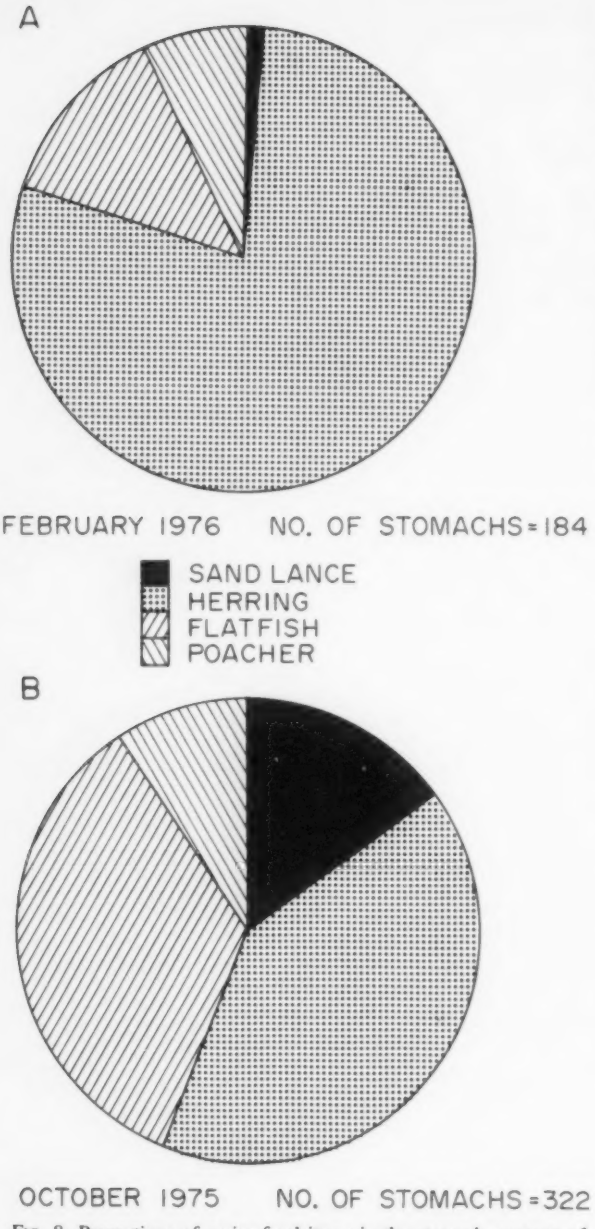

FIG. 8. Proportions of major food items in the stomach contents of adult Pacific cod collected in Hecate Strait (plotted from Westrheim 1977, table 17).

may be due to sand lance being an alternative forage species (Westrheim et al. 1989).

Regarding physical factors, only transport combined with stock size could predict the 1985 year-class. Temperature at hatching could still be an overriding factor if the range of winter temperatures extended to slightly higher levels as might be experienced in a general warming trend. The most southerly cod stock supporting a commercial fishery is the La Perouse stock off southwest Vancouver Island where February sea-surface temperatures sometimes reach between 9 and 10°C, about 2° higher than in Hecate Strait. The effects of these temperatures on survival rates of eggs and larvae might have some bearing on why this is the most southerly stock. Future investigations should continue to examine both factors and develop hypotheses regarding how they could influence other life stages and subsequently recruitment. Further observations would likely illuminate relationships between the two variables and how they affect recruitment. To investigate the effects of transport directly, one should try to observe larval and early juvenile cod being carried north and away from the area occupied by the population.

The implication for fishery stock assessment is that recruitment overfishing is likely to occur when standardized catch rates are less than 400 kg/h, the stock index level where recruitment production is maximized. Because of the apparent independence between the effects of stock size and transport, it should be possible to develop a yield model that addresses recruitment overfishing through regulations that promote maintenance of a minimal spawning biomass. Still it would be best for stock assessments to verify the model's estimates of year-class strength with a direct measure of prerecruit abundance prior to implementing catch limitations.

Acknowledgements

We thank R. P. Foucher and D. M. Ware for reviewing a draft of this paper and for making suggestions that improved it.

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Appendix

The model with a single independent variable (x_1) has the form

(1) $y = a + b_1x_1 + c_1x_2^2$

where a, b_1, and c_1 are fitted parameters and y is the dependent variable, catch-per-unit-effort of age-3 recruits, number of fish per hour. When an additional independent variable (x_2) is added to the model, y is then expressed as a response surface by the function

(2) $y = a + b_1x_1 + c_1x_1^2 + b_2x_2 + c_2x_2^2 + c_3x_1x_2$

where b_2, c_2, and c_3 are additional fitted parameters. Transformed variables are necessary for the statistical fit with the likelihood statistic (Schnute and McKinnell 1984)

(3) $\eta^\gamma = p + q_1\,\xi_1 + r_{1,1}\,\xi_1^2 + r_{1,2}\,\xi_1\,\xi_2 + q_2\,\xi_2 + r_{2,2}\,\xi_2^2.$

The relationship between η and y is

$$(4)\quad \eta = \frac{2y^\gamma - V^\gamma - v^\gamma}{V^\gamma - v^\gamma}.$$

The relationship between ξ and x_i, where $i = 1, 2$, indexes the independent variables

$$(5)\quad \xi_i = \frac{2x_i^{\alpha_i} - U_i^{\alpha_i} - u_i^{\alpha_i}}{U_i^{\alpha_i} - u_i^{\alpha_i}}.$$

The procedure for using the equation, for example to calculate a predicted recruitment from a set of the independent variables, is to transform each x_i to ξ_i (eq.5), calculate η (eq.3) using the parameters given below, and then solve for y (eq.2). The model is unstable for x_i values outside the range of x_i's used to estimate the parameters. We used this equation to predict the year-classes as age-3 recruits from 1982 to 1985, inclusive.

For the model with the independent variables of transport (x_1) and adult stock size (x_2), the values of the parameters were $\alpha_1 = 0.8774$, $\alpha_2 = 0.2302$, $\gamma = -0.4087$, $p = 0.0506$, $q_1 = -0.7343$, $q_2 = -0.4386$, $r_{1,1} = -0.1207$, $r_{1,2} = -0.3451$, $r_{2,2} = -0.5440$, $V = 357$, $v = 26$, $U_1 = 411.3$, $u_1 = 394.0$, $U_2 = 1646$, and $u_2 = 226$ where the maximum and minimum values are, respectively, V, v for catch-per-unit-effort for age-3 recruits, U_1, u_1 for the transport index, and U_2, u_2 for the adult stock size index.

Use of Zooplankton to Assess the Movement and Distribution of Alewife (*Alosa pseudoharengus*) in South-Central Lake Ontario in Spring[1]

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O'Gorman, R., E. L. Mills, and J. DeGisi. 1991. Use of zooplankton to assess the movement and distribution of alewife (*Alosa pseudoharengus*) in south-central Lake Ontario in spring. Can. J. Fish. Aquat. Sci. 48: 2250–2257.

Data from assessments of fish and zooplankton conducted during April and May–June 1986–88 in south-central Lake Ontario were examined for evidence that zooplankton size structure can be used to follow the movement of alewife (*Alosa pseudoharengus*). The spring influx of alewife into nearshore waters was linked with water temperature and coincided with a decline in the mean length of crustacean zooplankton and the virtual disappearance of zooplankters ≥0.9 mm. Alewife moving inshore to spawn fed heavily on the largest zooplankters, negating the possibility that changes in zooplankton size were wholly a response to seasonal recruitment as waters warm and the composition shifts to *Bosmina*. Offshore, there was usually no significant ($P < 0.05$) change in mean lengths of zooplankton in the upper water column between April and May–June, and zooplankters ≥0.9 mm always remained abundant, suggesting that few alewife were there from April through mid-June. We conclude that in large freshwater lakes where a planktivore is abundant, yet spatially concentrated, changes in size of crustacean zooplankton can facilitate understanding of the fish's movement and distribution.

A partir de relevés de poisson zooplancton et de zooplancton effectués en avril et en mai–juin 1986–1988 dans le centre-sud du lac Ontario, on a cherché à démontrer que la structure de taille du zooplancton peut server à suivre les déplacements du gaspareau (*Alosa pseudoharengus*). L'afflux de gaspareaux près de la rive était liée à la température de l'eau et coïncidait avec une diminution de la longueur moyenne des crustacés planctoniques et la quasi-disparition des organismes de 0,9 mm et plus. Le gaspareau qui s'approche de la rive pour frayer se nourrit largement du plancton le plus gros, ce qui élimine la possibilité que la taille du zooplancton change uniquement à cause du recrutement saisonnier, quand les eaux se réchauffent et que la composition favorise *Bosmia*. au large, il n'y avait généralement pas de changement significatif ($P < 0,05$) de la longueur moyenne du zooplancton dans le haut de la colonne d'eau entre avril et mai–juin, et les organismes ≥0,9 mmétaient toujours abondants, ce qui donne à penser qu'il y avait là peu de gaspareaux durant cette période. Nous concluons que dans les grands lacs d'eau douce où un planctivore abonde, mais localement, les changements de taille des crustacés planctoniques peuvent faciliter la compréhension des mouvements et de la distribution du poisson.

Received July 19, 1989
Accepted June 6, 1991
(JA233)

Reçu le 19 juillet 1989
Accepté le 6 juin 1991

Many studies have demonstrated that planktivorous fishes can affect the structure of lacustrine zooplankton communities (e.g. Brooks and Dodson 1965; Galbraith 1967; Wells 1970; Siegfried 1987; Mills and Forney 1988). The extent and timing of the planktivore's influence on the zooplankton community depend not only on the abundance of the fish but also on the species, and life stage because many fishes are plantivorous only when young. Nonanadromous alewife (*Alosa pseudoharengus*) are generally planktivorous throughout their life cycle in freshwater lakes (Odell 1934; Morsell and Norden 1968; Lackey 1969; Wells 1980) and they also are highly size-selective feeders (Brooks 1968; Hutchinson 1971; Janssen 1976; Kohler and Ney 1980). This feeding style of alewife combined with their ability to rapidly attain numerical dominance in the pelagia is probably the reason why they have been shown to structure the zooplankton communities of lakes (Wells 1970; Warshaw 1972; Kohler and Ney 1980, 1981).

Recent studies by Mills and Schiavone (1982) and Mills et al. (1987) illustrated the usefulness of zooplankton size in assessing community structure of fish populations. In the lakes they studied, however, the effects of spatial concentration of planktivorous fishes were unlikely to show up for two reasons: the lakes were physically and thermally homogeneous due to their relatively small size (surface areas 9 to 20 700 ha), and planktivory was diffused through the lakes due to the presence of many species of fish (each with a unique temporal distribution and habitat preference which overlapped that of other species). As the size of a water body increases, so does the physical

[1]Contribution No. 777 of the National Fisheries Research Center – Great Lakes, U.S. Fish and Wildlife Service, Ann Arbor, MI 48105, USA, and Contribution No. 104 of the Cornell Biological Field Station, Bridgeport, NY 13030, USA.

complexity and thermal diversity; large heterogeneous systems with an abundant planktivore provide the opportunity for spatial concentration of planktivory. In Lake George (New York), for example, which is 51 km long and has distinct north and south basins, the size structure of the zooplankton community in the south basin indicated intense planktivory by fish whereas that in the north basin indicated light planktivory; this difference led Siegfried (1987) to conclude that the rainbow smelt (*Osmerus mordax*) population was concentrated in the south basin.

In the Great Lakes, however, alewife are not distributed homogeneously throughout the water bodies during all seasons. In Lakes Michigan and Ontario, they overwinter in deep water (Wells 1968; Bergstedt and O'Gorman 1989). In southeastern Lake Michigan, Wells (1968) demonstrated that alewife numbers increase in the inshore waters during spring as mature alewife move there to spawn. Graham (1956) described a similar spring movement of alewife into the inshore waters of northern Lake Ontario from personal observations and interviews with commercial fishermen. This shoreward migration could have a profound impact on inshore food resources, particularly zooplankton.

Alewife were the predominant planktivorous fish in the main basin of Lake Ontario during 1980–88 (O'Gorman et al. 1987; Oswego Biological Station, unpubl. data). Intense size-selective planktivory should shift to different areas seasonally coincident with their annual migrations. If so, it should be possible to follow alewife movements from data on zooplankton size structure. Support for this idea comes from southeastern Lake Michigan (1971–77) where low spring densities of larger zooplankters inshore were hypothesized to be related to the spring shoreward movement of alewife and other planktivorous fishes (Evans et al. 1980).

The objectives of this study were to document the spring influx of alewife into the nearshore waters of south-central Lake Ontario, to describe the changes in the crustacean zooplankton community coincident with this movement, and to determine whether information on zooplankton size can advance our knowledge of alewife distribution and movement. Our approach was to use data on zooplankton and fish from annual assesments conducted during April–June in south-central Lake Ontario. Changes in alewife distribution were compared with body size and species composition of crustacean zooplankton during 1986–88. To help determine if size-selective planktivory by alewife was responsible for changes in the nearshore zooplankton community, we compared the size of zooplankters in alewife stomachs nearshore with the size of zooplankton in nearby waters during June 1988.

Materials and Methods

The study area was off the southern shore of Lake Ontario between Olcott and Sodus Bay, New York (a straight-line distance of 144 km). Within this stretch of shore, we netted fish along seven transects and collected zooplankton at five sites during 1986–88 (Fig. 1). We collected samples once in early spring, 20–30 April, and again in late spring, 25 May – 16 June. In any one year, all sampling in May–June was completed within a 2-wk period.

Zooplankton

Zooplankton collections were made at sites about 2 and 20 km from shore in the eastern and western regions of the study area. In each region, one site nearshore (2 km) and one site offshore (20 km) were visited during each sampling period. A distance of 18 km separating nearshore and offshore sites was considered sufficient to assume that zooplankton communities in these areas were spatially distinct. Collections in the east were made off Smoky Point over bottom depths of 22 and 206 m (Fig. 1). With one exception, collections in the west were made off Oak Orchard; bottom depths at the sites were 25 and 196 m. During May–June 1986, the nearshore collection in the west was made near Thirty Mile Point (about 29 km west of Oak Orchard), where the bottom depth was 25 m.

We collected zooplankton in vertical tows with a conical plankton net 0.5 m in diameter, 2 m in length, and constructed of 153-μm mesh. Before each plankton haul, we determined temperatures in the water column with a bathythermograph. Nearshore, the net was lowered to the bottom except when thermal stratification was strong (June 1987); we then lowered the net to the top of the thermocline (about half way to tbe bottom). Offshore, sampling was to a depth of 50 m except in June 1987 and 1988 (Oak Orchard only) when a shallow thermocline formed and we sampled to depths of 11 m or less. Retrieval was hand-over-hand. The zooplankton was preserved in a sugar–formalin solution (Haney and Hall 1973) immediately after being rinsed from the net.

In the laboratory, the entire sample was examined to find the large zooplankter *Bythotrephes cederstroemi*. Individual zooplankters were then identified to species (Edmondson 1963; Balcer et al. 1984), enumerated, and measured in one to three 1-mL subsamples. Immature zooplankters (neonates and copepodites) were also counted and measured in all subsamples. Zooplankters were measured from the anterior margin of the head to the base of either the tail spine (in cladocerans) or the caudal rami (in copepods). Counts and lengths were recorded with electronic calipers interfaced with an Apple[2] computer (Mills and Confer 1986).

Zooplankter lengths at nearshore and offshore sites in each month were rank transformed and an analysis of variance (ANOVA) was performed on the ranks. Although the result of a rank transformation followed by ANOVA is only conditionally distribution free, it is robust. The true level of significance is usually close to the approximate level of significance used in the test, no matter what the underlying population distribution might be (Conover 1980). If the F value indicated a significant difference ($P < 0.05$) between mean lengths, Tukey's studentized range test was used to determine which means were different. We compared length–frequency distributions of zooplankters by use of the Kolmogorov–Smirnoff test (Conover 1980).

Fish

The distribution of alewife was determined with catches from two cooperative surveys conducted annually by the U.S. Fish and Wildlife Service (USFWS) and the New York Department of Environmental Conservation (NYDEC). Survey methodology is described in detail in O'Gorman and Schneider (1986) and O'Gorman et al. (1987).

Briefly, fish were sampled with a 12-m (headrope) bottom trawl equipped with a cod end constructed of 9-mm-mesh (stretched measure) knotless nylon. During each sampling

[2]Use of trade names does not constitute U.S. Government endorsement of commercial products.

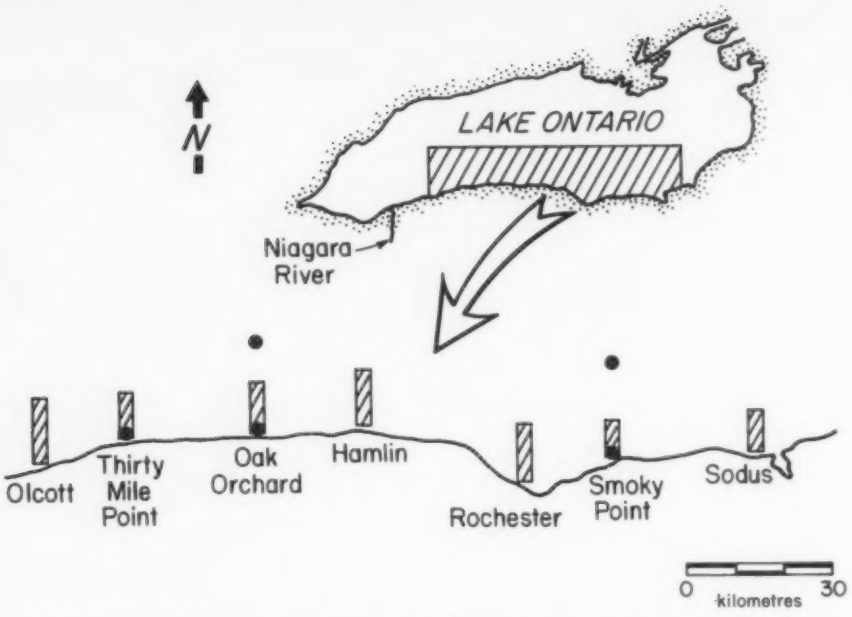

FIG. 1. Locations of areas sampled with bottom trawls (bars) and plankton nets (circles) in Lake Ontario, 1986–88.

TABLE 1. Depths of bottom trawling sites at seven transects in south-central Lake Ontario.

Standard trawling depth (m)	Distance from shore (km)	
	Mean	Range
15	1.5	1.0–2.3
25	2.6	1.8–4.7
35	3.3	2.2–6.6
45	4.0	2.8–8.3
55	4.7	3.3–9.2
65	5.7	4.0–10.8
75	6.5	4.4–11.9
85	7.0	5.4–10.4
95	7.3	5.8–9.9
110	8.6	6.5–13.4
130	10.1	7.4–14.2
150	12.4	10.3–13.6

period, a total of seven transects were fished in south-central Lake Ontario by the R/V *Kaho* (USFWS) and the R/V *Seth Green* (NYDEC) using standardized, matched trawling procedures (Fig. 1) except that only four transects were fished in April 1986 (Olcott, Thirty Mile Point, Rochester, and Smoky Point). At each transect there were 12 possible standard trawling depths between 15 and 150 m (Table 1). However, not all of the deeper standard depths were fished each time a transect was sampled (see below). The trawling sites were between 1 and 14 km from shore, and two thirds were less than 7 km from shore (Table 1).

Our measure of the distribution of alewife was percent of the catch taken at the standard depths in each sampling period (Fig. 2). Percent of catch was mass of alewife (all life stages combined) caught per unit of effort (per trawl haul; CPUE) at each standard depth, divided by the sum of the CPUE's from all depths in that sampling period. When computing CPUE, we combined catches at 85 and 95 m as well as those at 110, 130, and 150 m because few trawl tows were made at some of these standard depths in May–June.

Temperature (degrees Celsius) of the water near bottom was determined with a bathythermograph either before or after nearly all trawl hauls. Mean temperature of the water near bottom in the study area during each sampling period was calculated by averaging the mean temperatures at the standard trawling depths. Here, too, we combined data from 85 and 95 m as well as data from the three deepest standard depths.

Although fish were assessed on bottom and zooplankton were assessed in the water column, nearshore in spring, alewife are mostly near bottom during the day whereas at night they make vertical migrations to feed (Brandt 1980; Janssen and Brandt 1980). We would have preferred to track the movement of alewife 20 km from shore but bottom trawl assessments were not routinely conducted in this area because the bottom was beyond the depth range of alewife as well as other fishes. Furthermore, we lacked a midwater trawl to sample at middepths.

We examined the stomach contents of six yearling alewife (mean total length 92 mm, range 85–99 mm) and 13 adult alewife (164 mm, 145–184 mm) caught at 15 m (2.3 km from shore) off Rochester during the June 1988 cooperative survey. Shortly after capture, we punctured the coelomic cavities of the alewife and immersed them in a solution of 10% formalin.

In the laboratory, we examined only the contents of the anterior (dorsal) limb of the stomach because the contents of the posterior (ventral) limb were usually digested. Any macroinvertebrates in the anterior limb were removed, counted, and identified. Water was then added to the remaining microinvertebrate fraction to bring the total volume to 20–40 mL. After mixing, a Henson–Stemple pipette was used to withdraw two or more 1-mL subsamples. Intact zooplankters in the subsam-

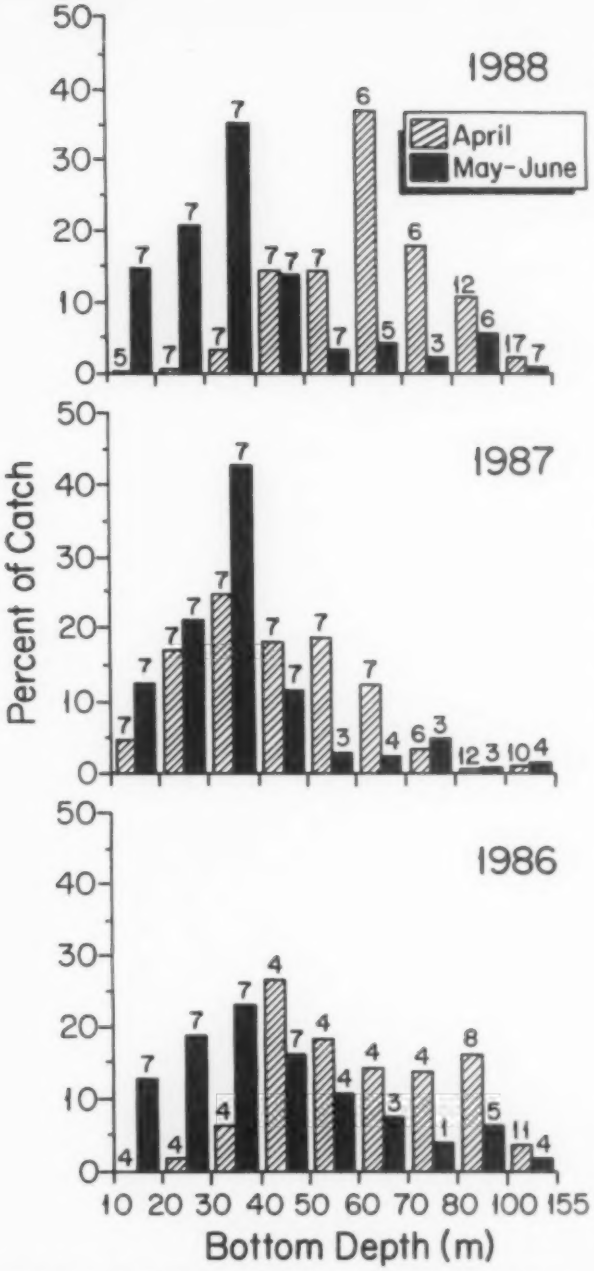

FIG. 2. Depth distribution of alewife in south-central Lake Ontario during April and May–June 1986–88. Numbers of trawl hauls made in the various depth intervals are shown above each bar.

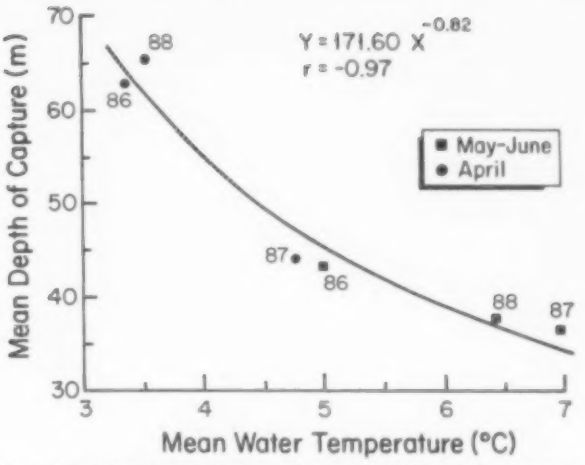

FIG. 3. Relationship of the mean depths at which alewife were captured to the mean temperatures of water near bottom during two time periods, 1986–88, Lake Ontario.

ples were identified, counted, and measured in exactly the same manner as zooplankters in the subsamples from the plankton-net catches (see above). When zooplankters or macroinvertebrates were not intact, we counted single or paired body parts (such as tailspines or eyes) as whole organisms.

We compared mean lengths of zooplankters in alewife stomachs with the mean lengths of zooplankters 2 km from shore by use of a nested ANOVA (SAS 1988) of the rank transformed lengths and Tukey's studentized range test. For this analysis, we separated yearling from adult alewife because life stage (size) might affect what zooplankters were eaten.

Results

Lake Temperature

Lake Ontario was much warmer in 1987 than in 1986 or 1988. In April, bottom waters at our trawling sites were mostly <4°C except in 1987 when they were always >4°C. In May–June, bottom waters were frequently >5°C except in 1986 when they were seldom that warm. Between the two sampling periods, the mean temperature of water near bottom increased 1.6°C in 1986, 2.2°C in 1987, and 1.9°C in 1988. In both sampling periods the mean water temperature was highest in 1987 and lowest in 1986.

At the two nearshore zooplankton collecting sites, surface waters in April averaged 5.8°C in 1987 compared with about 3.9°C in other years. By June, surface waters averaged 18.5°C in 1987 but only 14.0°C in 1986 and 11.9°C in 1988. At the offshore sites, surface waters in April averaged 4.1°C in 1987 but only 2.5°C in 1986 and 3.3°C in 1988. Corresponding temperatures in May–June were 14.6, 4.7, and 5.7°C.

Distribution of Alewife

Alewife in south-central Lake Ontario invariably occupied greater depths in April than in May–June (Fig. 2). Also, their depth distribution varied more among years in April than in May–June. Mean depths of capture in early spring were 63 m in 1986, 44 m in 1987, and 65 m in 1988 whereas in late spring the corresponding mean depths of capture were 43, 37, and 38 m. Mean depths of capture were negatively related to mean temperatures of near-bottom water (Fig. 3). Percent of the catch taken at 15 and 25 m (the shallowest depths fished) rose from 2% in April to 31% in May–June 1986, from 22 to 37% in 1987, and from 1 to 36% in 1988.

Structure of the Zooplankton Community

We identified 15 species of crustacean zooplankton over the 3-yr study but only a few species were ever abundant. The

TABLE 2. Mean lengths (mm) of crustacean zooplankton 2 and 20 km from shore at two locations in south-central Lake Ontario, April and May–June 1986–88. For each location and year, mean lengths followed by the same letter are not different statistically ($P > 0.05$, ANOVA of rank-transformed lengths followed by Tukey's studentized range test). Numbers of zooplankters measured are shown in Fig. 4.

Sampling period	Location and year					
	Oak Orchard			Smoky Point		
	1986[a]	1987	1988	1986	1987	1988
			20 km			
April	0.85 z	0.59 z	0.74 z	0.83 z	0.68 z	0.74 y
May–June	0.88 z	0.58 z	0.56 y	0.87 z	0.66 z	0.78 y
			2 km			
April	0.75 y	0.58 z	0.83 z	0.84 z	0.63 z	0.91 z
May–June	0.74 y	0.28 y	0.50 y	0.52 y	0.24 y	0.45 x

[a]The nearshore sample in May–June was collected 29 km west of Oak Orchard.

European cladoceran *B. cederstroemi*, which was recently reported from Lake Ontario by Lange and Cap (1986), was not seen in our plankton-net samples.

In 1986 and 1988 during April and May–June, the two most abundant species of zooplankton nearshore and offshore were *Diacyclops thomasi*, which predominated, and *Leptodiaptomus minutus*. Together they comprised 84–98% of the zooplankton exclusive of nauplii. In April 1987, a dominant species was lacking and zooplankton was mostly (>90%) a mixture of *D. thomasi*, *Acanthocyclops vernalis*, *L. minutus*, *Skistodiaptomus oregonensis*, and (nearshore) *Bosmina longirostris*. By June 1987, about 80% of the zooplankters nearshore were *B. longirostris* whereas offshore about 80% were *A. vernalis* (Smoky Point) or *D. thomasi* (Oak Orchard).

Three species of *Daphnia* occurred infrequently in our samples. *Daphnia retrocurva* was the only daphnid found in all 1987 nearshore samples. *Daphnia galeata mendotae* occurred offshore near Oak Orchard in April 1986 and 1988 and in June 1987 as well as at nearshore sites in May–June 1986 (Thirty Mile Point) and 1988 (Oak Orchard). A single specimen of *Daphnia pulex* was found nearshore in April 1988 (Oak Orchard).

In general, mean lengths of zooplankters at nearshore sites were not significantly different ($P < 0.05$) from those at offshore sites in April but were significaatly shorter than those at offshore sites in June (Table 2). At the offshore sites, there was usually no statistically significant change in mean lengths of zooplankton between April and May–June. In contrast, mean lengths of zooplankton nearshore in May–June were significantly shorter than in April except during 1986 in the western reaches of the study area; that May–June sample, however, was collected at Thirty Mile Point, 45 km west of the mouth of the Niagara River. At all sites, mean lengths were usually longest in 1986 and shortest in 1987.

The seasonal increase of smaller zooplankters between April and June as nearshore waters warmed coincided with the virtual disappearance of the largest zooplankters. Consequently, nearshore length distributions of zooplankton in May–June were significantly different ($P < 0.01$) from those in April (Fig. 4). The density (numbers per square metre) of plankters ≤0.4 mm nearshore was always much higher in May–June than in April whereas the density of plankters ≥1.1 mm declined to 0, or nearly so, in May–June. Similarly, plankers 0.9–1.0 mm long had usually disappeared from the nearshore zone by May–June, and when they were still present, their density was reduced from that in April. At the western site in 1986, where zooplankton mean size and length distribution nearshore did not differ between early and late spring, the densities of small and large plankters in May–June were greater than in April (Fig. 4). Offshore, the seasonal increase of smaller zooplankters was delayed by the cooler water and densities of small plankters in May–June were usually similar to those in April. Large zooplankters were present offshore in April and May–June, and their densities were similar in both sampling periods. As a result, mean lengths of zooplankton and length distributions of zooplankton in offshore waters in late spring rarely differed from those in early spring (Table 2; Fig. 4). The only exception was at Oak Orchard in 1988 where there was a disproportionate increase in smaller zooplankton between sampling periods.

Zooplankton Prey of Alewife

Adult and yearling alewife fed exclusively on zooplankton off Rochester in June 1988 and the bulk of the zooplankton eaten was cyclopoid copepods (mostly *D. thomasi*). The frequency of occurrence of zooplankters in adult alewife was 82% cyclopoid copepods and 6% *B. cederstroemi*. Yearling alewife ate mainly (94%) cyclopoid copepods.

Alewife were eating the largest zooplankton available along the south shore of Lake Ontario in June (Fig. 5). Mean length of zooplankton in stomachs of adult and yearling alewife caught 2.3 km from shore was significantly larger ($P < 0.05$) than the mean length of zooplankton caught in plankton nets 2 km from shore. Although the mean length of zooplankton eaten by the adults was greater than the mean length of zooplankton eaten by the yearlings, the mean lengths were not significantly different ($P > 0.05$) from each other. Plankters eaten by adult alewife averaged 0.71 mm and those eaten by yearling alewife averaged 0.68 mm whereas plankters in the net samples averaged 0.47 mm. We could not measure the large-bodied *B. cederstroemi* eaten by adult alewife because they were partially digested. If we had been able to measure them, however, there would have been a much larger difference between the mean size of plankters in adult alewife stomachs and in the net samples (*B. cederstroemi* were never found in our net samples, most likely because of their low abundance and good mobility which allowed them to avoid the slow-moving net).

Discussion

The seasonal movements of alewife in the Great Lakes have been described largely from catches with bottom trawls (Wells 1968; Reigle 1969a, 1969b). Despite evidence that many alewife are pelagic (Brown 1972; Argyle 1982; Olson et al. 1988), sampling of middepths has been sporadic, particularly offshore where the bottom can be beyond their depth range. Consequently, the seasonal distribution of alewife off bottom is poorly known, especially in offshore waters (but see Bergstedt and O'Gorman (1989) for a description of the alewife's distribution in autumn and winter in southeastern Lake Ontario). Alewife are also size-selective planktivores, and shifts in the zooplankton community have been associated with alewife predation (Wells 1970; Evans 1986; Evans and Jude 1986; Warren et al.

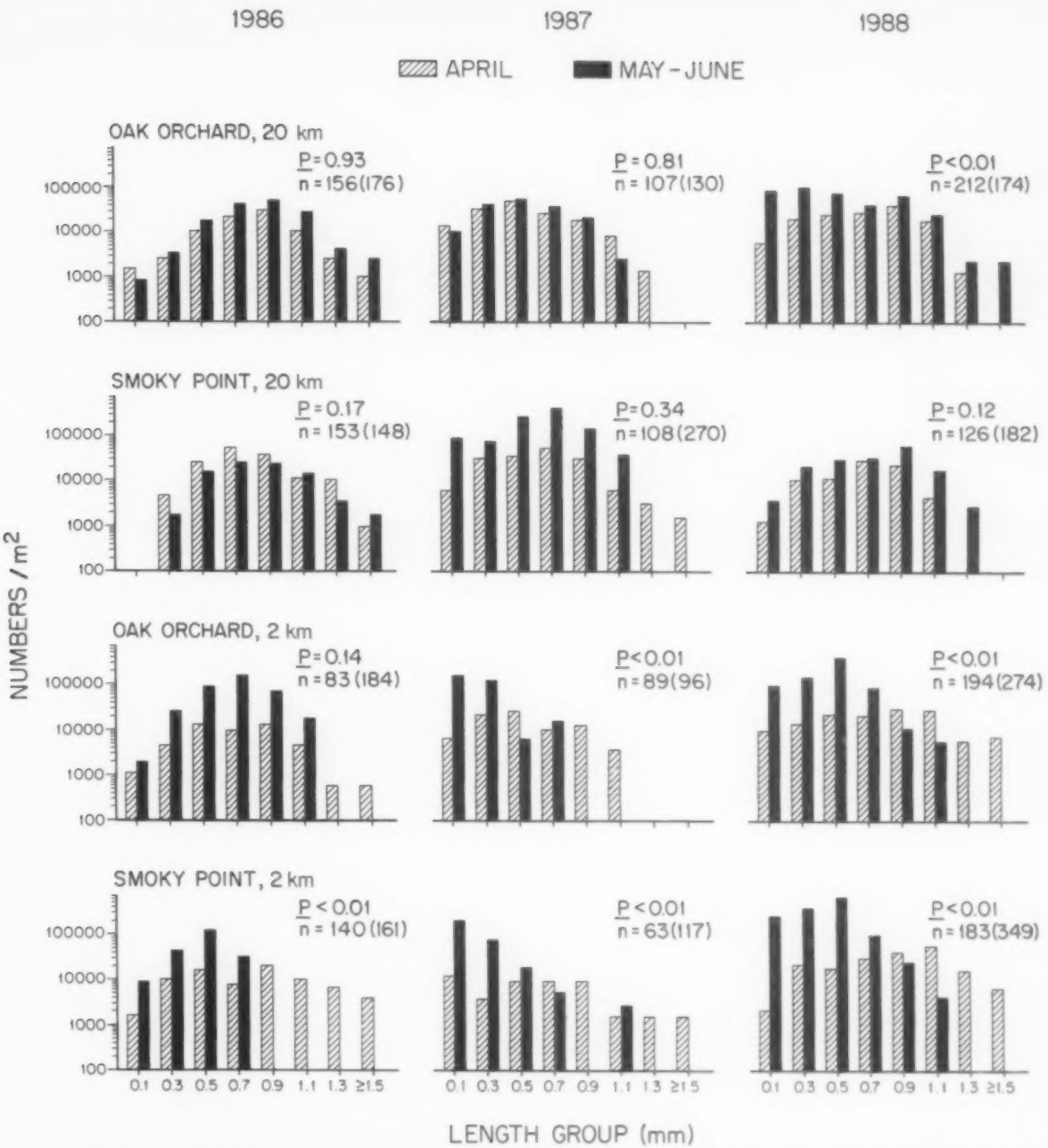

FIG. 4. Length–frequency distribution of crustacean zooplankton 2 and 20 km from shore at two locations in south-central Lake Ontario, April and May–June 1986–88. Above each histogram is the *P* value for the Kolomogorov–Smirnov test and the number of zooplankters measured in April and (in parentheses) May–June.

1986; Hewett and Stewart 1989). Because of the potential use of zooplankton in describing fish community structure (Galbraith 1975; Mills and Schiavone 1982; Mills et al. 1987), changes in the zooplankton community could help bridge the information gap on alewife distribution and movement. In Lake Ontario, our results not only confirm that alewife influence the size structure of the zooplankton community but also support our contention that changes in body length of the crustacean zooplankton community can be useful in assessing alewife seasonal distribution and movement.

In nearshore Lake Ontario, although the decline in mean size of zooplankton coincided with the spring shoreward migration of alewife, we cannot rule out the possibility that the April–June decrease in zooplankton body length was in response to

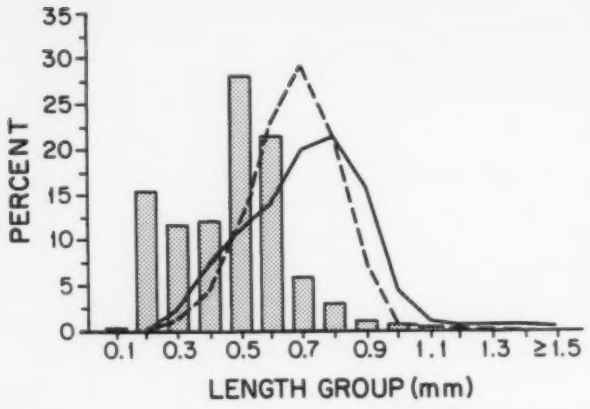

FIG. 5. Length–frequency distribution of crustacean zooplankton in the water column over bottom depths of 22–25 m, 2 km from shore (stippled bars, Oak Orchard and Smoky Point combined) and in stomachs of adult (solid line) and yearling (broken line) alewife caught with a bottom travel at 15 m, 2.3 km from shore near Rochester, NY, Lake Ontario, June 1988. The numbers of zooplankters measured were 274 from Oak Orchard and 349 from Smoky Point, 1295 from adult alewife, 1042 from yearling alewife.

the seasonal recruitment of young zooplankton that occurs each spring as waters warm. In May–June, species composition of nearshore zooplankton differed slightly from that offshore probably due to the more rapid warming of nearshore water and the concomitant earlier start of the annual cycle of species succession, as described by Patalas (1969), Watson and Carpenter (1974), Czaika (1978), and Johannsson et al. (1985). For the same reason, rapid warming of the entire lake in 1987 probably caused the species mixture in 1987 to differ from that in 1986 and 1988. However, the notion that changes in zooplankton size structure were wholly in response to nearshore warming can be dispelled by the fact that alewife moving inshore to spawn fed heavily on the largest zooplankters. The mean size of zooplankton in nearshore waters was greatly reduced between April and June; by June, zooplankters ≥0.9 mm were rare. In the absence of alewife predation during May–June, a decline in zooplankton size is possible but a broad size spectrum of zooplankters ranging from neonates to large adults would be expected (Mills and Schiavone 1982). When warming triggered reproduction offshore at Oak Orchard in 1988, mean size declined but large zooplankters remained abundant. Other than this, the large mean size of offshore zooplankton did not decline by May–June. Zooplankters ≥0.9 mm were always abundant offshore. This leads us to believe that few alewife occupied the upper 50 m of the water column during April–June and that zooplanktivory was not intense. Finally, with the loss of the alewife in the offshore waters of Lake Michigan, large cladocerans such as *Daphnia pulicaria* and *B. cederstroemi* have expanded and persisted through spring – early summer warming (Evans and Jude 1986; Scavia et al. 1986; Lehman 1987), lessening the possibility that changes in zooplankton size nearshore were most reflective of seasonal warming.

The vertical and horizontal distribution of alewife in southern Lake Ontario during spring was clearly governed by the temperature of the lake; the lower the temperaure, the deeper and farther from shore alewife were concentrated. In winter, alewife spatially concentrated in deep water and are located in depths from 35 to 110 m in southeastern Lake Ontario (Bergstedt and O'Gorman 1989). In the spring, the shoreward movement of alewife coincides with a decline in mean length of crustacean zooplankton and a virtual disappearance of zooplankton ≥0.9 mm. Consequently, water temperature is linked with alewife distribution and movement, and wherever these fish are spatially concentrated, their planktivory has a significant effect on the zooplankton community. As a result, alewife do not have the same effect on the zooplankton community in different areas of the lake during the same season which strengthens the idea of using zooplankton to track fish distribution and movement. On the negative side for the alewife population, offshore reserves of large-bodied zooplankton are available as a food source in spring, yet the bulk of the alewife are inshore. Because of their effect on nearshore zooplankton, intense competition for food resources may depress alewife growth and recruitment and possibly the growth and recruitment of other fish species (Smith 1970; Crowder 1980; O'Gorman et al. 1987).

Although size of zooplankton clearly changed in response to the springtime shift in the distribution of alewife, there was one instance when there was no change in nearshore zooplankton size between April and May–June. In 1986, the nearshore May–June sample was collected at Thirty Mile Point, about 45 km east of the mouth of the Niagara River, instead of at the usual site near Oak Orchard, about 74 km east of the river mouth. We suspect that, in this case, the Lake Ontario zooplankton community was influenced by zooplankton washing out of Lake Erie (currents in this area of the lake are quite strong and usually flow easterly along the south shore (Saylor et al. 1981)). Patalas (1969) noted that in June the outflow of the Niagara River enriched the local zooplankton community. This suggests that the springtime restructuring of nearshore zooplankton by alewife predation can be mitigated by local water masses entering Lake Ontario.

For those involved in the management of fish populations in the Great Lakes, information on the abundance, distribution, and movement of prey fishes is a continuing need. This study clearly indicates that in large freshwater lakes where a planktivore is abundant, yet spatially concentrated, the size of crustacean zooplankton can provide a useful index of the prey species movement and distribution. Because of this indexing quality, it is likely that information on zooplankton size in large, cold-water lakes could also be linked to changes in abundance, condition, and growth of planktivorous prey species. If true, the use of zooplankton could have broad applications to management and assessment of prey species.

Acknowledgments

This study was supported by funds from the U.S. Fish and Wildlife Service, the New York State Department of Environmental Conservation, and Cornell University. Special recognition is given to Mark Prout, Ruth Sherman, Clinton Wiltse, and Don Widzowski for analyzing the zooplankton samples.

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Stable Isotopes of Sulfur, Carbon, and Nitrogen as Indicators of Trophic Level and Fish Migration in the Lower Mackenzie River Basin, Canada

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Stable isotopes of sulfur, carbon, and nitrogen were used to investigate the incorporation of nutrients into food chains (fish, invertebrates, and plants) and the influence of migration on the nutritional origins of fish in two freshwater systems in the Mackenzie Delta region. Sulfur isotope analyses of fish muscle showed that broad whitefish (*Coregonus nasus*) in Travaillant Lake and lake whitefish (*Coregonus clupeaformis*) in the Kukjuktuk Creek system were migrant populations which had grown mostly on sources outside the local food base. A marine food source was indicated for the lake whitefish, while no specific source was determined for the broad whitefish. The $\delta^{15}N$ clearly defined the trophic levels in the local food chains in both systems. Fish were in three levels in Travaillant Lake. Amphipods were in a level below the lowest fish and sediments and macrophytes were in the lowest level. The Kukjuktuk Creek fish were in two levels above a level including macrophytes, amphipods, and zooplankton. The $\delta^{13}C$ varied widely even within single fish species local to Travaillant Lake. The $\delta^{13}C$ did not differentiate migrants from local fish. Plants of both the C3 and C4 photosynthetic pathway were indicated by carbon isotopes.

On a utilisé des isotopes stables de soufre, de carbone et d'azote pour étudier l'assimilation de bioéléments dans une chaîne alimentaire (poissons, invertébrés et plantes) et l'influence de la migration sur les origines alimentaires des poissons de deux réseaux hydrographiques de la région du delta du Mackenzie. Des analyses des teneurs en isotope de soufre dans certains tissus musculaires de poisson ont révélé que le corégone tschir (*Coregonus nasus*) du lac Travaillant et le grand corégone (*Coregonus clupeaformis*) du réseau du ruisseau Kukjuktuk appartiennent à des populations migratrices et que leurs principales sources de nourriture sont situées à l'extérieur de ces systèmes. La principale source de nourriture du grand corégone est d'origine marine, mais pour le corégone tschir, aucune source précise n'a pu être identifiée. Le $\delta^{15}N$ a nettement caractérisé les niveaux trophiques de la chaîne alimentaire locale des deux réseaux. Dans le cas du lac Travaillant, les poissons se répartissaient en trois niveaux. Les amphipodes se retrouvaient à un niveau inférieur au plus bas niveau des poissons, tandis que les sédiments et les macrophytes formaient le plus bas niveau. Les poissons du ruisseau Kukjuktuk se répartissaient en deux niveaux au-dessus d'un niveau comprenant des macrophytes, des amphipodes et du zooplancton. La teneur en $\delta^{13}C$ variait beaucoup, même chez une même espèce de poisson sédentaire du lac Travaillant. Cet isotope n'a pas permis de distinguer les poissons migrateurs des poissons sédentaires. Les isotopes du carbone ont permis d'identifier les plantes appartenant aux types photosynthétiques C3 et C4.

Received November 26, 1990
Accepted May 1, 1991
(JA818)

Reçu le 26 novembre 1990
Accepté le 1 mai 1991

The stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) have been recently used with increasing frequency to describe food web relationships and food sources (reviewed by Peterson and Fry 1987). Sulfur isotopes ($\delta^{34}S$) have received less attention in this context because the methods are more difficult, sulfur is less abundant in organic matter, and sulfur dioxide has the reputation of being "dirty" in mass spectrometers. Given certain geographic and geochemical combinations, sulfur isotopes can be extremely valuable in discriminating between food sources (for example, see Petersen et al. 1986). In highly complex systems like the Mackenzie River, its tributaries, the Delta lakes and channels, and the Beaufort Sea coastal lake systems, we have found sulfur isotopes, as a third component, to be very valuable in interpreting food webs leading to the production of fish. This study details the isotopic data from components of the food webs of two lakes in the lower Mackenzie River – Beaufort Sea region of northwestern Canada. This is part of a larger study of the energy flow to fish and their migration in this system.

Study Area

Travaillant Lake is a large lake (115 km^2, maximum depth >30 m) located on a tributary of the Mackenzie River (Fig. 1), the Travaillant River, which enters about 150 km upstream of Arctic Red River. The drainage area upstream of Travaillant Lake is approximately 2000 km^2, annual precipitation is about

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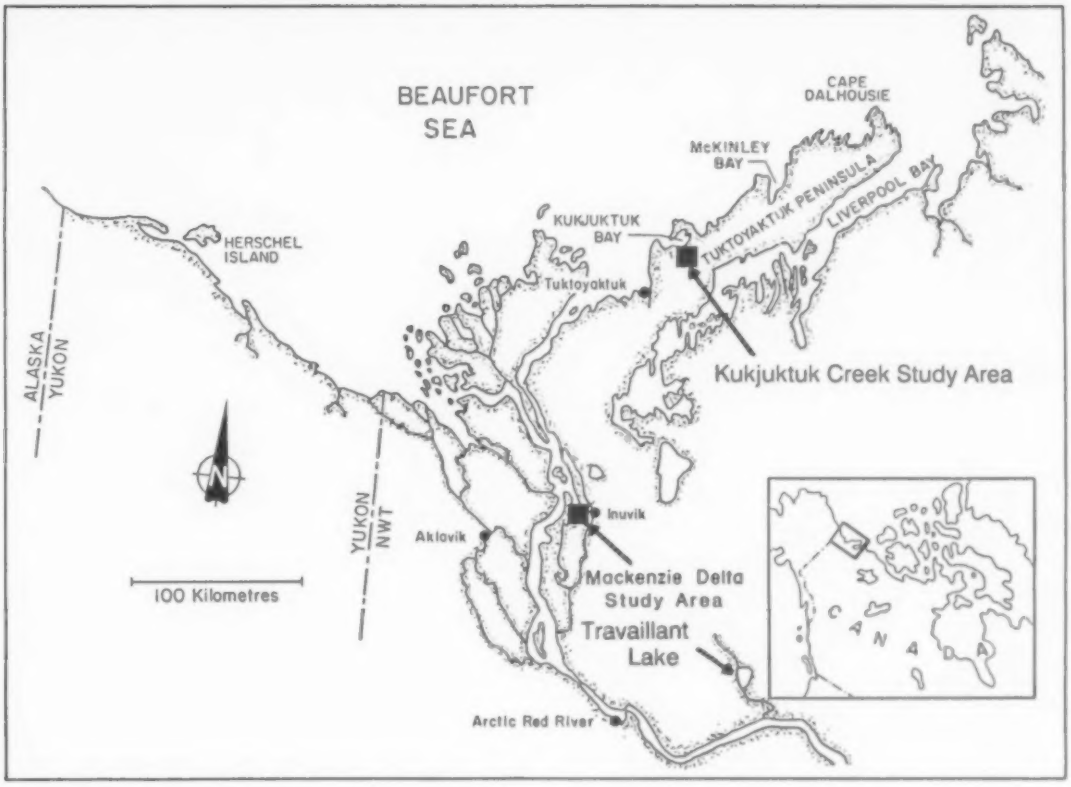

FIG. 1. Travaillant Lake and Kukjuktuk Creek study areas in the Mackenzie Delta region.

TABLE 1. Typical chemical composition of water from Travaillant Lake and Lake 18 (Kukjuktuk Creek system), N.W.T., Canada (na = not available).

Parameter (units)	Travaillant L., Sept. 16, 1989	Lake 18, 1986[a]
Total dissolved N (μg/L)	290	298
Total dissolved P (μg/L)	2	10
Suspended N (μg/L)	58	90
Suspended P (μg/L)	8	12
Suspended C (μg/L)	695	na
Dissolved inorganic (μmol/L)	1250	1418
Dissolved organic C (μmol/L)	570	414
pH	8.33	8.3
Alkalinity (μequiv/L)	1140	1268
Sodium (mg/L)	3.24	6.1
Potassium (mg/L)	1.29	1.1
Magnesium (mg/L)	5.00	5.6
Calcium (mg/L)	16.7	19.6
Chloride (mg/L)	2.15	12.1
Sulfate (mg/L)	5.96	1.3
Conductivity (μS/cm)	133	175
Chlorophyll *a* (μg/L)	0.9	4.3

[a]Means from Anema et al. (1990b, table 6).

30 cm, and evapotranspiration is about 15 cm (Fisheries and Environment 1978). The lake supports a domestic fishery, primarily for broad whitefish (*Coregonus nasus*). Basic water chemistry for Travaillant Lake is given in Table 1. This lake was chosen for the study because fish collections were available at low cost from other studies (Reist 1987), it had a diverse fish community, and it contained broad whitefish which were thought to migrate to and from the Mackenzie River. It is representative of tributary systems near to or east of the lower Mackenzie Valley.

Lake 18 (69°30′30″N, 132°28′00″W) is one of approximately 81 lakes in the Kukjuktuk Creek system on the Tuktoyaktuk Peninsula about 23 km northeast of the village of Tuktoyaktuk. Lake 18 has an area of 283 ha, a maximum depth of 5.2 m, a mean depth of 1.5 m, and a water renewal time of about 1.4 yr. The lake and its carbon budget have been examined in some detail (P. Ramlal, Freshwater Institute, Winnipeg, Man., pers. comm.) and phytoplankton productivity has been described by Fee et al. (1988). Detailed water chemistry is available (Anema et al. 1990a, 1990b), and a typical summer set of values has been given in Table 1 for comparison with Travaillant Lake. The outflow of Kukjuktuk Creek enters Kukjuktuk Bay, in the Beaufort Sea. Lake 18 was chosen for the study because detailed limnologic and fisheries information and samples of fishes and other biological components were available.

The lower Mackenzie River system is characterized by extensive spawning migrations of certain species such as the broad whitefish and lake whitefish (*Coregonus clupeaformis*) although there may be populations which do not migrate. The migration of these species has been documented by several authors (Chang-Kue and Jessop 1983, 1991; Bond and Erickson 1985; Lawrence et al. 1984; and others). In addition to their

spawning migrations, these fishes move extensively during their early life, using the creeks and lakes of the Tuktoyaktuk Peninsula, the Delta lakes and channels, or coastal bays for feeding or overwintering. The patterns of movement of broad whitefish have been elegantly described using radiotagged fish (Chang-Kue and Jessop 1983) as well as by standard mark–recapture and seasonal catch techniques (summarized by Reist and Bond 1988). However, little is known about the feeding behaviour of these fish during migration or of the group and site fidelity from year to year.

Hesslein et al. (1991) have described the distribution of stable isotopes of sulfur and carbon in broad whitefish in relation to their migration in the lower Mackenzie River basin. They found about half of the broad whitfish caught in spawning runs to have isotopic signatures similar to those expected from feeding in the Delta lakes or lakes on the Tuktoyaktuk Peninsula. Large numbers of fish were also found to have isotopic compositions intermediate to those values, suggesting some feeding in both locations. Many fish were also found to have isotopic signatures which could not be assigned to a known location, suggesting that there are other important feeding locations in the area.

Broad whitefish and lake whitefish caught in the outer Delta and Tuktoyaktuk Harbour, probably during migrations, typically have a high percentage of empty stomachs. In broad whitefish, 70–95% of the stomachs were empty (Percy 1975; Jones and Den Beste 1977; Bond 1982; Lawrence et al. 1984). Both species had fewer empty stomachs and fuller stomachs when caught in lakes on the Tuktoyaktuk Peninsula and in the Delta (Bond and Erickson 1985). While the migrant whitefish use these lakes for intensive feeding (Bond and Erickson 1982; Chan-Kue and Jessop 1991), there are also significant local populations of fish. These include piscivores as well as those with invertebrate diets. Northern pike (*Esox lucius*), lake trout (*Salvelinus namaycush*) in deeper lakes, least cisco (*Coregonus sardinella*), white sucker (*Catostomus commersoni*), and ninespine stickleback (*Pungitius pungitius*) are often present.

Methods

The principle behind the use of stable isotopes for the investigation of food chains is that the isotopic ratio of an element is indicative of a source or location and then may (1) remain unchanged as the element moves through the food web, preserving the record of the origin (sulfur is used for this and carbon has also been used in this way), (2) change in a constant manner through each trophic transfer (nitrogen acts like this), or (3) change as a result of different biochemistry (carbon acts like this in the C3 and C4 photosynthetic pathways; all three elements can be influenced by bacterial reduction, for example). The three elements are essential dietary components derived from the consumption of organic matter except by plant synthesis.

Analytical methods used to determine isotope ratios have been described in some detail by Hesslein et al. (1989) and will be described only briefly here. Approximately 1 g of dry tissue was required for sulfur isotope analyses, while carbon and nitrogen were analyzed on a single aliquot of about 15 mg dry weight. The methods and locations of the fish catches are described elsewhere (Reist 1987). Fish from the Kukjuktuk Creek system were caught both in Lake 18 and in the creek flowing toward Kukjuktuk Bay. All fish from Travaillant Lake were caught in the lake. Dorsal muscle was taken from all but the smallest fish, in which case the whole fish was homogenized. Sediment samples were taken from the top of a core from Lake 18 and Kukjuktuk Bay and from an Eckman dredge in Travaillant Lake. These were soaked in warm dilute HCl to remove any carbonate material. Aliquots of plankton were taken from net hauls using 73-μm mesh and screened at 200 μm. Amphipods were combined to make up the weight required. Macrophytes were ground and subsampled. Total inorganic carbon (acidified and degassed) and sulfate (Hesslein et al. 1988) were also analyzed. Biological samples for sulfur isotopic analyses were decomposed to sulfate by nitric acid digestion, nitrate fusion, and barium precipitation, followed by thermal decomposition to sulfur dioxide. Carbon and nitrogen samples were dried, decomposed to nitrogen and carbon dioxide by a modified Dumas method, and cryogenically separated and trapped.

All isotopic determinations were performed on a dual inlet isotope ratio mass spectrometer (VG Micromass 602E). Standards used for sulfur are traceable to Canyon Diabolo, carbon to PDB, and nitrogen was standardized against air. Over several years of operation, the typical reproducibility of determinations has been at 2 standard deviations: 0.3‰ for sulfur, 0.1‰ for carbon, and 0.4‰ for nitrogen.

Results and Discussion

Travaillant Lake

The sulfur isotopes of the fish in Travaillant Lake fell into two groups: broad whitefish, over the range from −10.7 to −15.8‰, and all other species in the range −8.2 to −10.2‰ (Fig. 2). Sulfate in lake water had a value of −6.8‰ and the sediments were −6.1‰. The range of sulfur isotope values in fish other than broad whitefish (−8.2 to −10.2‰) is interpreted as representing the local food base in which sulfur is fixed into amino acids. The values for broad whitefish indicate that they are visitors which have assimilated a significant fraction of their sulfur from other food bases. It is not possible that their flesh was produed from sulfur-containing amino acids from Travaillant Lake. No significant differences exist in the three years of catches (1984, 1985, and 1986) of broad whitefish although the five most negative sulfur values are from the 1985 and 1986 catches. If the broad whitefish had been feeding in Travaillant Lake at the time of capture, we would have expected to see the sulfur isotope signal move toward that of the local species (Fig. 2) as sulfur is metabolically added or replaced in the flesh. This may explain the large variation in the broad whitefish $\delta^{34}S$. Since neither the origin of $\delta^{34}S$ nor the variance within the whole population to which these migrant fish belong is known, there is too much uncertainty to be able to assess the change in signal which may have occurred by feeding in Travaillant Lake.

The nitrogen isotopes in the local populations of Travaillant Lake show very clearly the levels in the food chain structure. Grouping the fish on the basis of $\delta^{15}N$ (Fig. 2) places all the white suckers, ninespine stickleback, and small northern pike (young-of-the year (yoy) and one 280-g fish) in the lowest level (6.3–9.3‰), lake whitefish, least cisco, and smaller northern pike (661–1259 g) in the second level (9.3–12.3‰), and the two largest northern pike (1299 and 1332 g) and all the lake trout in the highest level group (12.3–15‰). There are no data for the stomach contents of fish from Travaillant Lake, but the general food components of the above species in the Mackenzie

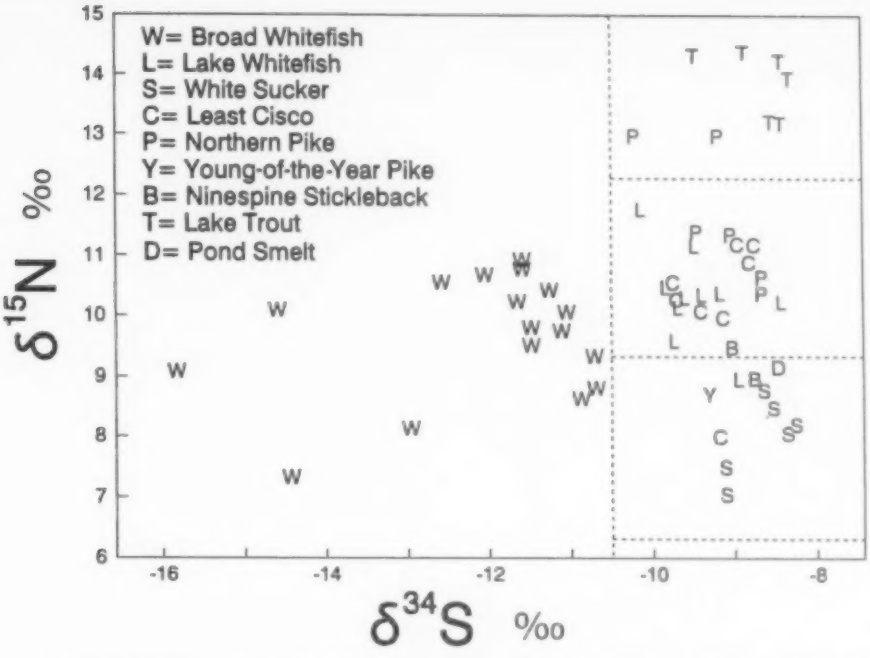

FIG. 2. Sulfur and nitrogen isotopes in Travaillant Lake. The broken vertical line delineates local and migrant fish. The broken horizontal lines delineate levels in the food chain (see text).

River basin are known (Hatfield et al. 1972a, 1972b; Strange 1985). Ninespine stickleback and white suckers feed on insect larvae and detrital matter, while yoy northern pike feed on insects and plankton. The $\delta^{15}N$ data suggest that herbivores must be a major component of the diet of this group. This is further suggested by the $\delta^{15}N$ of the species in the next level. Lake whitefish in this area feed on corixids, which can be herbivorous or predacious (Hungerford 1948), and molluscs, which filter feed on plankton (pelecypods) or graze on attached algal communities (gastropods). Either the predatory invertebrates dominate the lake whitefish diet or we must conclude that the diet of the species in the group with a lower $\delta^{15}N$ level described above contains a lot of plant nitrogen. Least cisco feed on plecopterans which are often predacious and tricopterans which can be either predators or herbivores. Hatfield et al. (1972a, 1972b) identified >90% of least cisco stomach contents as "terrestrial insects"; however, other studies have reported few terrestrial insects in whitefish or cisco (W. Bond, Department of Fisheries and Oceans, Winnipeg, Man., pers. comm.). The northern pike in the middle $\delta^{15}N$ level (661–1259 g) are generally omnivorous and opportunistic feeders. The diet may include fish, molluscs, insects, or crustaceans. In Travaillant Lake, these small northern pike could not have been feeding on least cisco or lake whitefish of the size range analyzed, since they shared the same $\delta^{15}N$ level. Feeding on white suckers, ninespine stickleback, other small fish, or predatory insects is certainly possible. The lake trout and the two largest northern pike (1299 and 1332 g) are most likely piscivores feeding on the lake whitefish, least cisco, or smaller northern pike. Significant feeding on white suckers is unlikely, as the $\delta^{15}N$ difference is too large. Significant feeding on the migrant broad whitefish is also not possible, as the $\delta^{34}S$ signal of the predators is not outside the Travaillant Lake range. Broad whitefish generally feed on molluscs, amphipods, and corixids (Hatfield et al. 1972a, 1972b; Bond and Erickson 1985), but the meaning of the $\delta^{15}N$ signal in these fish cannot be interpreted in the context of the Travaillant Lake system, as the sulfur isotopes clearly indicate an outside source of protein.

If we ignore the broad whitefish, which we have classified as migrants, the $\delta^{13}C$ and $\delta^{15}N$ (Fig. 3) do allow us to show the position of other components in the food chain for which an insufficient amount of sample was available for sulfur isotope analyses. A sample of benthic invertebrates (mostly amphipods picked from a dredge haul) were in the level below the lowest fish, as defined by $\delta^{15}N$. Their $\delta^{13}C$ is slightly less negative than the lowest in the fish. A macrophyte sample consisting largely of Potamogeton (species not identified) has slightly lower $\delta^{15}N$ than the invertebrates and much less negative $\delta^{13}C$ than anything else in Travaillant lake. It is possible that the Potamogeton use the C4 photosynthetic pathway and fractionate only about 11‰ from their carbon dioxide source ($\delta^{13}C$ of the dissolved inorganic carbon was −5.2‰). The sediment carbon does not seem to be influenced by this macrophyte carbon, as noncarbonate carbon in the sediment sample had a $\delta^{13}C$ value of −27.4‰. Its $\delta^{15}N$ value was 3.7‰, near values observed in plant material. It is possible that the more positive $\delta^{13}C$ value of the macrophytes is partly responsible for the wide range in the $\delta^{13}C$ values from the biota.

The more classical isotopic approach to food web analyses using the $\delta^{13}C$ and $\delta^{15}N$ gives a less clear picture of the levels within the web (Fig. 3). Because the $\delta^{13}C$ values of the broad whitefish show almost the same distribution as the local Travaillant Lake fish, but cover the entire two lower levels of $\delta^{15}N$, the interpretation of nitrogen would have been confused. There is no indication from the $\delta^{13}C$ that the broad whitefish are from another site and food source, as made clear by the

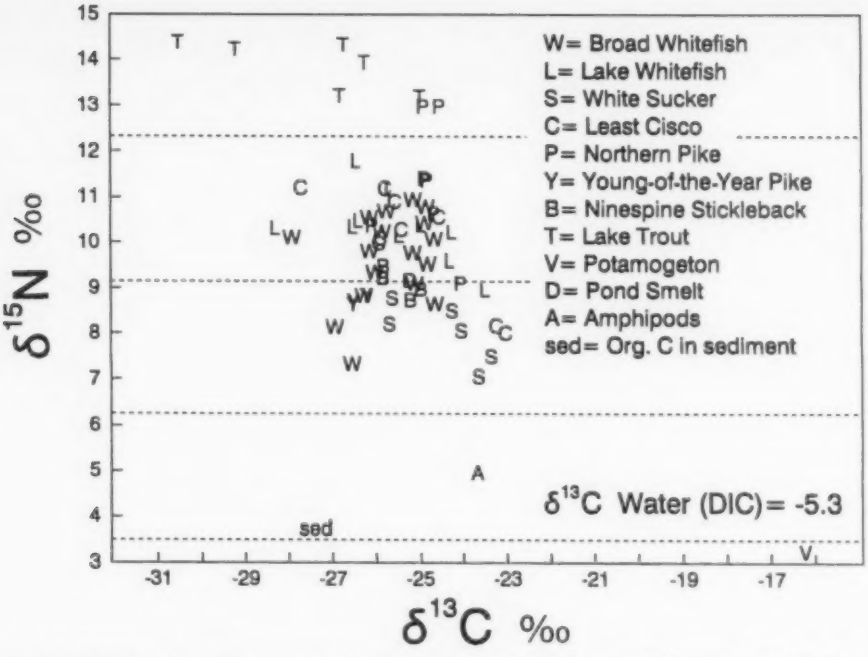

FIG. 3. Carbon and nitrogen isotopes in Travaillant Lake. The broken lines delineate levels in the food chain (see text).

sulfur isotope values. In other studies, $\delta^{13}C$ alone has been used to distinguish between food sources.

Fractionation of $\delta^{13}C$ during food assimilation is generally expected to be about 1‰, becoming more positive at each step (De Niro and Epstein 1978). The observation in the Travaillant Lake biota is in the opposite direction. Lake trout are as much as 7‰ more negative than white suckers. Even without the extreme values, there appears to be a negative correlation between $\delta^{13}C$ and $\delta^{15}N$. There is no immediately apparent explanation for this trend. Differences between species cannot be invoked, as there is a 6‰ range among the lake trout and nearly 5‰ among lake whitefish. Variation in feeding habits between individuals could explain the differences if there was a food source within Travaillant Lake with $\delta^{13}C$ in the range of −31‰. However, no such $\delta^{13}C$ value has been found in any samples to date. The differences in lake trout are not consistent with size or sex. Perhaps physiological differences such as lipid contents could be responsible, but the range is very large for physiological effects. There is no trend in the $\delta^{13}C$ with $\delta^{34}S$ among the lake resident populations.

Kukjuktuk Creek System (Lake 18)

The sulfur isotopes in fishes from the Kukjuktuk Creek system fell in the very wide range of −18 to +13‰ (Fig. 4). Macrophytes, sediments, and sulfate from lake 18 were in the range of −17 to −9‰. We assign the range −18 to −11‰ to fish grown on the local food base and assume that fish with sulfur isotopes in the −10 to +13‰ range have had their diet influenced by other food sources. Six of the several lake whitefish sampled had $\delta^{34}S$ in the range of +7 to +13‰. Seven of the 18 broad whitefish were in the intermediate range of −10 to +3‰. Among broad whitefish, there is no distinction that can be made between fish caught in downstream or upstream traps or in Lake 18. Size does not allow any discrimination between fish of different $\delta^{34}S$ values.

The interpretation of nitrogen isotopes even within the local sulfur signal is not as clear as in Travaillant Lake, although the general structure is the same. Again, for some components of the food chain, insufficient sample was available for $\delta^{34}S$ analyses, but $\delta^{15}N$ and $\delta^{13}C$ analyses were possible. The $\delta^{15}N$ for these samples are shown with $\delta^{13}C$ (Fig. 5). For this interpretation we have assumed that the invertebrates and the macrophytes are of local origin. The northern pike clearly occupy the top level ($\delta^{15}N$ of 12–14‰) and show little variance in $\delta^{15}N$ or $\delta^{34}S$. Most broad whitefish were in the next $\delta^{15}N$ level (8–11‰), but two broad whitefish were 7.8 and 6.7‰. zooplankton (net haul, 73 μm, screened at 200 μm) and amphipods were in the 5–8‰ range. The macrophytes pose something of a problem in that their $\delta^{15}N$ values cover the same range as those of the zooplankton and amphipods. This suggests that the invertebrates do not derive their nitrogen from the macrophytes. *Tolypella intricata* grows only in a small area of the deepest portion of the Lake 18 and may not be an important part of the carbon budget. *Lemna tricsula* is free floating but has an undetermined productivity and also may not be significant in the food chain. The moss grows under 1–3 m of water and constitutes a large biomass. The $\delta^{15}N$ of the moss (mixed community of several species; Ramlal et al. 1991) samples varied considerably. This may be a result of the limited mixing conditions of the water within the moss layer which can be up to 10 cm thick. Perhaps much nitrogen recycling occurs in this layer. It is not known whether moss is incorporated into the food chain leading to fish. Where then does the sediment nitrogen with an $\delta^{15}N$ value of 3.5‰ come from, and can it provide a sufficient contribution to support the food chain? Photosynthesis studies

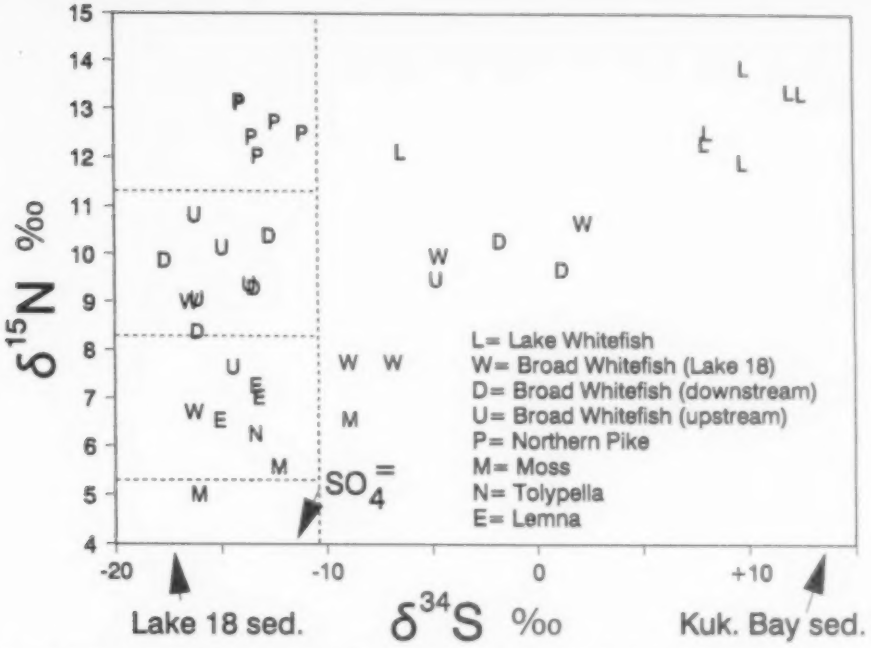

FIG. 4. Sulfur and nitrogen isotopes in the Kukjuktuk Creek system. The broken vertical line delineates local and migrant fish. The broken horizontal lines delineate levels in the food chain (see text).

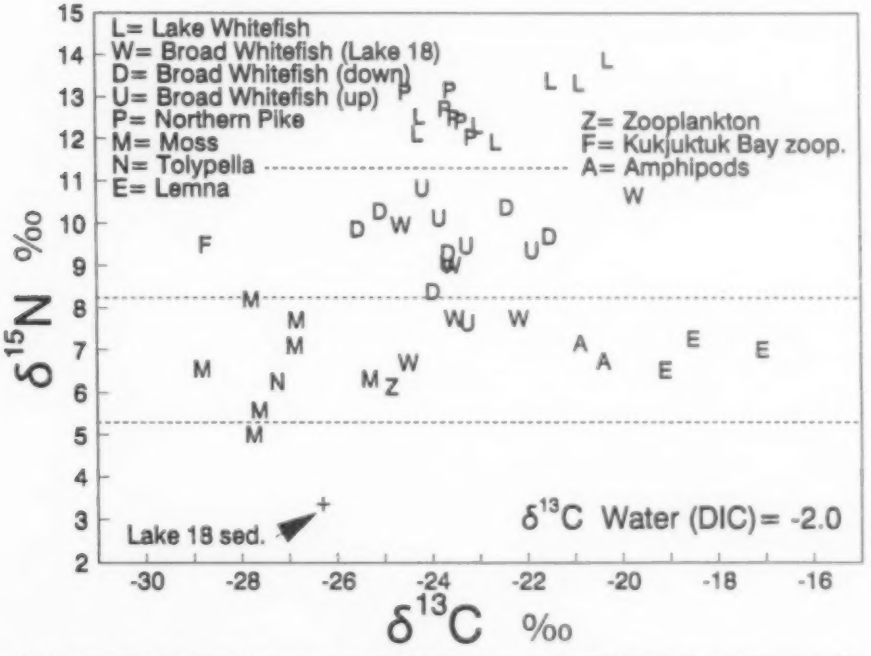

FIG. 5. Carbon and nitrogen isotopes in the Kukjuktuk Creek system. The broken lines delineate levels in the food chain (see text).

using oxygen production in boxes placed on the sediments (P. Ramalal, pers. comm.) showed high rates in the 0- to 1-m depth zone, even though no large amount of biomass was evident at the sediment surface. It is possible that the productivity of the algal coating on sediments and moss is the major component of the primary link in the food chain. This would satisfy the food chain $\delta^{15}N$ evidence, but leaves the question of why the other plant $\delta^{15}N$ is at the higher level observed.

The northern pike in Lake 18 had $\delta^{15}N$ values that would allow them to have been feeding on the broad whitefish, but not on the lake whitefish, because the $\delta^{15}N$ of the lake whitefish is the same as that of the northern pike and the $\delta^{34}S$ is vastly different. This may be explained by the observation that these lakes are used by large numbers of broad whitefish <200 mm but few lake whitefish that small (Bond and Erikson 1982; Chang-Kue and Jessop 1991). We believe that the lake whitefish $\delta^{15}N$ and $\delta^{34}S$ reflect an influence from marine food sources. Ocean sulfate is +21‰ and $\delta^{15}N$ values in ocean food chains which we have measured in this area are higher than in freshwater for fishes with similar food preferences (R. H. Hesslein, unpubl. data). A surface sediment sample from Kukjuktuk Bay, into which the Kukjuktuk Creek flows, had $\delta^{34}S$ of 13.0‰, just the maximum found in the lake whitefish. Zooplankton from Kukjuktuk Bay had an $\delta^{15}N$ value of 9.5‰, which fits very well as food for the lake whitefish at 11.5–14.0‰. The carbon from the Kukjuktuk Bay zooplankton was −28.7‰, much lower than that in the lake whitefish. Carbon and nitrogen in the Kukjuktuk Bay sediment were, respectively, −23.8 and 1.5‰. Lake whitefish, especially those <225 mm, are thought to spend significant periods of time in the coastal waters of the Tuktoyaktuk Peninsula (Bond 1982; Lawrence et al. 1984). This scenario of marine feeding followed by upstream migration, based on the stable isotope data, is entirely consistent with the life history description of lake whitefish given for this area by Chang-kue and Jessop (1991), based on tag data. They suggested that most lake whitefish live in coastal waters for 4–6 yr before beginning an annual migratory stage to the lakes on the Tuktoyaktuk Peninsula. However, there are some old lake whitefish which had resided in the lakes for a period of years. All the lake whitefish shown in Fig. 4 and 5 were in the size range of 290–385 mm, which would translate to an estimated age of 6–11 yr (Bond 1982).

The isotopic data for broad whitefish are also entirely consistent with the life history description given by Chang-Kue and Jessop (1991). They reported that young broad whitefish (<2 yr old and <200 mm) migrate up Kukjuktuk Creek (over 1 million in 1979) and remain in the lakes 1–4 yr, after which most fish begin an annual open-water migration pattern. The authors also found that there are older broad whitefish (>400 mm, 8–13 yr old) that spend 1 yr or more in the lakes. The broad whitefish in Fig. 4 and 5 were of two size ranges: 295–315 mm (4–5 yr) and 385–480 mm (8–15 yr). Only one of the five smaller fish had a $\delta^{34}S$ value outside the range we assigned to local Lake 18 fish.

The $\delta^{13}C$ analyses for the Kukjuktuk Creek system show, as in Travaillant Lake, two widely different food sources, both of which seem to influence the upper trophic levels (Fig. 5). The carbon fixed by the moss *Tolypella* and in the zooplankton is in the range of −25 to −29‰, while th $\delta^{13}C$ in *Lemna* is −19 to −17‰ and in the amphipods is −21 to −20‰. Again, as in Travaillant Lake, sediments appear to be dominated by carbon from the lower source at −28 to −26‰. Dissolved inorganic carbon was −1.2‰.

Conclusions

While there is certainly much information about food chains which can be elucidated by stable isotopes of carbon and nitrogen, the use of stable isotopes of sulfur greatly clarifies the feeding relationships in complex estuarine systems like the Mackenzie Delta which contain migrating and anadromous fish populations. This is in marked contrast with studies of food chains in the open ocean or Canadian Shield lakes (Hesslein et al. 1988) where sulfur isotopes are almost constant due to mixing or domination by atmospheric sources, respectively. Sulfur is particularly useful as a source tracer because amino acids containing sulfur are essential to animals, thus effectively eliminating fractionation in the food chain.

This study has described two situations in which the use of carbon and nitrogen isotopes would have led to erroneous or ambiguous interpretations of food chain relationships. Furthermore, some concern must be expressed about the use of stable carbon isotopes from only whole flesh samples as indicators of carbon sources. This concern arises from the broad range of values among individuals in populations such as the lake trout of Travaillant Lake. While we cannot categorically exclude the possibility of different carbon sources for individual fish, we feel that it is highly unlikely. At the same time, we cannot explain the broad range of $\delta^{13}C$ values observed.

Estuarine, anadromous, and coastal fisheries are of great importance to Canada. Because of the wide varieties of habitats available to fishes in these systems and the great mobilities of some of the species, it has been difficult to develop confidence in the description of the feeding requirements and locales of the fish stocks. Some innovative techniques like radiotagging (Chang-Kue and Jessop 1983) and genetic fidelity studies (Reist 1989) have begun to confirm the migration and reproductive behaviour. We feel that stable isotope studies of sulfur, carbon, and nitrogen powerfully complement such information and will aid in developing a much more complete understanding of food chains and feeding habits.

Acknowledgements

We would like to thank Dr. J. Reist and his various field and laboratory crews for supplying the fish and morophologic data for this study. Discussions with B. Fallis, B. Bond, K. Chang-Kue, D. Bodaly, and E. Jessop were essential to formulating the interpretation of these results. They also reviewed parts or all of the manuscript. Dr. R. Hecky and his field personnel in Inuvik collected the nonfish samples and identified the species. Funding was received from NOGAP project B3 and the Fisheries Joint Management Committee in addition to the institutional support of the Department of Fisheries and Oceans.

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A Critical Look at the Idea of Terminal Molt in Male Snow Crab (*Chionoecetes opilio*)

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Whether or not male snow crab (*Chionoecetes opilio*) undergo a terminal molt when they develop large chelae is reviewed. Evidence supporting terminal molt includes laboratory studies in which large-clawed animals failed to molt and field studies which failed to find large-clawed males with mouth parts indicating imminent molting. Both studies were poorly documented. The field studies were largely conducted during inappropriate seasons and molt status based on a questionable mouth part stage. There is also an unsubstantiated claim that large-clawed animals fail to form limb buds for limb regeneration during the next molt and an inconclusive study of the degeneration of the Y-organ. We present new data against terminal molt including capture of large-clawed males with internal carapaces indicating impending molts, capture of two large-clawed animals with mouth parts indicating active premolting, recovery of two tagged large-clawed animals which molted while at liberty, and that the largest small-clawed animals usually are not large enough to account (through growth in a single molt) for the largest of the large-clawed animals. Also, one large-clawed male reportedly molted in captivity. We conclude that the existence of a terminal molt in male snow crab is not proven.

On rexamine si le crabe des neiges mâle (*Chionoecetes opilio*) entreprend une mue finale lorsqu'il développe de grosses pinces. Les indices à l'appui d'une mue finale comprennent des travaux en laboratoire dans lesquelles des crabes à grosses pinces n'ont pas mué et des études sur le terrain qui n'ont pas réussi à trouver des mâles à grosses pinces aux pièces buccales indiquant une mue imminente. Ces études sont mal documentées; les travaux sur le terrain ont été surtout effectués durant les mauvaises saisons et l'état de mue se fondait sur un stade de pièce buccale mal défini. On trouve également l'affirmation non démontrée selon laquelle les animaux à grosses pinces ne forment pas de bourgeons pour regénérer les membres durant la mue suivante et une étude non concluante de la dégénérescence de l'organe Y. Nous présentons de nouvelles données réfutant la mue finale : la capture de mâles à grosses pinces à la carapace interne indiquant une mue imminente, la capture de deux mâles à grosses pinces aux pièces buccales indiquant une pré-mue active, la recapture de deux crabes à grosses pinces étiquetés qui ont mué dans la nature et le fait que que les plus gros animaux à petites pinces sont trop petits pour atteindre la taille des plus gros à grosses pinces en une seule mue. En outre, on signale la mue d'un mâle à grosses pinces en captivité. Nous concluons que la mue finale du crabe des neiges n'est pas prouvée.

Received November 8, 1990
Accepted June 6, 1991
(JA795)

Reçu le 8 novembre 1990
Accepté le 6 juin 1991

The idea that male snow crab (*Chionoecetes opilio*) undergo a terminal molt was suggested by Miller and O'Keefe (1981). Conan and Comeau (1986) claimed proof of a terminal molt and linked it with the development of large chelae. Donaldson and Johnson (1988) and Donaldson (1988) challenged these assertions and Conan et al. (1988a) and Conan et al. (1988b) defended the idea of a terminal molt. An international workshop on snow crab biology obtained a "consensus" that male *C. opilio* "are now considered to have a terminal molt" (Jamieson et al. 1988, p. viii). However, at least 7 of the 38 workshop participants remain unconvinced. Recently, Conan et al. (1990) reiterated their conviction of a terminal molt.

Terminal molt means, according to Conan and Comeau (1986, p. 1718), that "morphometrically-mature" (i.e. large-clawed) males "extremely seldom, if ever, molt." The alternative is that these males molt commonly although possibly less frequently than "morphometrically-immature" (i.e. small-clawed) males. We find the terms "morphometrically-mature" and "morphometrically-immature" confusing because the

relationships of male chela morphometry to sexual maturity and mating capability remain unclear (Ennis et al. 1988, 1990). Because of this confusion, we prefer to use the terms "large-clawed" and "small-clawed" when referring to chela morphometry, and we concentrate only on the question of whether large-clawed males can molt.

Intermolt period is known to increase with the size of the animal for a variety of crustacean species. Therefore, any comparison of molting frequency between large- and small-clawed males must account for sizes of the animals in order to attribute differences in molt frequency to claw size.

The question of whether or not terminal molt exists has important implications for stock assessment and fishery management. If only small-clawed animals molt, then the proportion of the sublegal-sized population with large claws will never reach the Canadian legal harvest size of 95 mm carapace width (cw). Variability in size at terminal molt would then be important for computing yield per recruit and also for determining the expected number of times a male mates during its life. Also, if terminal molt exists, data on age composition of carapaces could be used to estimate mortality of large-clawed animals by catch curve methods.

In this paper, we review the published evidence on terminal molt in male snow crab and present new data on the question. Emphasis is placed on determining the kinds of data, and the logical arguments, which will resolve the issue.

Statistical Treatment of Morphometric Data

A major portion of the first paper on terminal molt in male snow crab (Conan and Comeau 1986) was devoted to a principal component analysis and linear discriminant function analysis. This analysis appears to have been misunderstood by a number of biologists who work on snow crabs. Conan and Comeau measured six size variables on crabs and computed the principal components on log-transformed data. When the data were plotted on the first two principal component axes, they formed two clouds of points. The investigators assigned each animal to a cloud, but it is not clear from their paper how they did this. They apparently assigned animals to groups according to whether the observation was either above or below the first princpal component axis (the abscissa) or an unspecificed diagonal line. Either way, the separation was arbitrary because there was no concomitant information (ground truth) with which to establish a biological basis for defining categories. Thus, animals "in the middle" were not assigned to groups on the basis of any biological or statistical argument; the method of assigning animals to groups did not allow for any overlap of the distributions. Conan and Comeau noted that when measurements from additional males, caught by divers while the animals were in the process of mating, were plotted on the first two principal component axes, all the animals fell into one cloud. They therefore suggested that these two clouds pertain to "mature" and "immature" animals. However, Ennis et al. (1990) have shown that males from both clouds are capable of mating successfully. Therefore, we prefer to avoid terms which relate the clouds to states of maturity and use instead the descriptive terms "large-clawed" and "small-clawed" because the two groups can essentially be obtained more easily by looking at the relationship between chela height and carapace width. Conan and Comeau then showed that, having assigned the animals to groups, they could duplicate the partition of the data with close to 100% accuracy using just two variables (chela height and carapace width). This shows only that four of the six size variables are redundant. Specifically, the discriminant function analysis does not confirm the validity of the groupings from the prinicpal component analysis. These two analyses succeed only in showing that there are two clouds or groups of animals (large-clawed and small-clawed). This result is obvious from a scatter plot of the data and, as acknowledged by Conan and Comeau, has been demonstrated by Powles (1968), Watson (1970), and Coulombe et al. (1985). The analysis also shows that a sample of breeding males from Bonne Bay, Newfoundland, fell in the cloud with large claws. It does not prove in any way that large-clawed animals are less likely to molt than small-clawed animals.

We note that an estimated misclassification rate for the discriminant function analysis pertains only to the reliability of the two-variable method for reproducing the arbitrary partition based on the six variables. Similarly, the calculation of the probability of an animal falling into a particular group, as described by Conan and Comeau (1986), pertains to the groups they subjectively defined. We stress the point only to make it clear that these methods do not provide objective, quantitative guidance in determining to which biological group a borderline data point has the greatest affinity.

Since Conan and Comeau's (1986) method of classification is complicated and essentially subjective, we have chosen to use a simpler subjective procedure which gives essentially the same results. We simply examine a scatter plot of chela height on carapace width and subjectively divide the data points into two clouds.

We also note that mixture analysis can be used to determine the probability that a data point falls in a given statistical group, where the statistical groups are defined as the components of a mixture of two normal (or lognormal) distributions (Warren 1990). If one could assume that these statistical populations represented biological groups, then one would have an objective method for separating animals using statistical principles (see Warren 1990 for an application of the method to a snow crab population).

Molting in Captivity

Conan and Comeau (1986) kept 110 large-clawed and 24 small-clawed males in the laboratory for 10 mo from November 1984 to September 1985. All animals were "of the same size range," but the sizes were not specified. The shell conditions of the experimental animals and details of the experimental conditions (holding facilities, density of animals, etc.) were not given. None of the 110 large-clawed males molted, while 16 of the 24 small-clawed males (67%) molted. If the large-clawed males were larger on average than the small-clawed males, then a smaller proportion of the large-clawed animals would be expected to molt than of the small-clawed animals if the intermolt period increases with size. Similarly, if the large-clawed group had a higher proportion of new-shelled animals than the small-clawed group, then one would expect a smaller proportion of the large-clawed group to molt. Finally, if large-clawed animals were held in more crowded conditions, or under conditions that were in some way less suitable, than the conditions under which the small-clawed crabs were held, then a smaller proportion of the large-clawed animals would be expected to molt. It is impossible to resolve these questions from Conan and Comeau's paper. However, it is worth noting that Conan et al. (1988a) and Conan et al. (1990) described

subsequent aquarium work in which an unspecified proportion of the animals died either within the first 6 mo of captivity or while in the process of molting. If large-clawed animals require more stringent environmental conditions for molting, then the potentially stressful conditions of captivity may have inhibited molting activity of the large-clawed animals.

Conan et al. (1988a) and Conan et al. (1990) reported similar results from additional aquarium studies. However, these reports again fail to describe the experimental subjects and conditions and hence the results cannot be evaluated.

Foyle (1987) kept 22 large-clawed male crabs in captivity for approximately 11 mo. One crab molted. Its premolt morphometry was 92.8 mm cw, 18 mm chela height, and 15.8 mm chela width. These data verify that it was clearly a member of the large-clawed group according to criteria supplied to Foyle by Conan.

Annual Trends in Carapace Condition in Nature

Donaldson (1988) observed that if terminal molt does not exist and if environmental conditions are unfavorable for molting in a given year (i.e. there is an unusually high incidence of "skip-molting"), then the proportion of old-shelled animals should rise; the following year, if environmental conditions return to normal, the old-shelled animals should molt and the proportion of old-shelled animals should decline. Conditions consistent with this scenario were observed from 1978 to 1981 and from 1985 to 1987 (Donaldson 1988; Dawe et al. 1990). Taylor et al. (1990) also noted that in some years in Newfoundland, molting activity is reduced and they attributed this to unusually low water temperatures. Thus, there is ample reason to believe that some years may have abnormally low incidences of molting. An alternative explanation for an increase in old-shelled animals in one year, followed by a decline in the next, is that there could be recruitment failure in the first year followed by normal or above-average recruitment in the next year. Thus, if the alternative explanation is true, one should be able to detect a trough in the size–frequency histogram or a gap in a scatter plot ("A" in Fig. 1) that will cause an elevated level of old-shelled animals in the following year at a size that is one molt larger than the size where the trough or gap was located ("B" in Fig. 1). Donaldson, however, did not present any size–frequency data to test this alternative explanation. For this sort of argument to be convincing, it would be necessary to examine the data by claw type (large-clawed versus small-clawed).

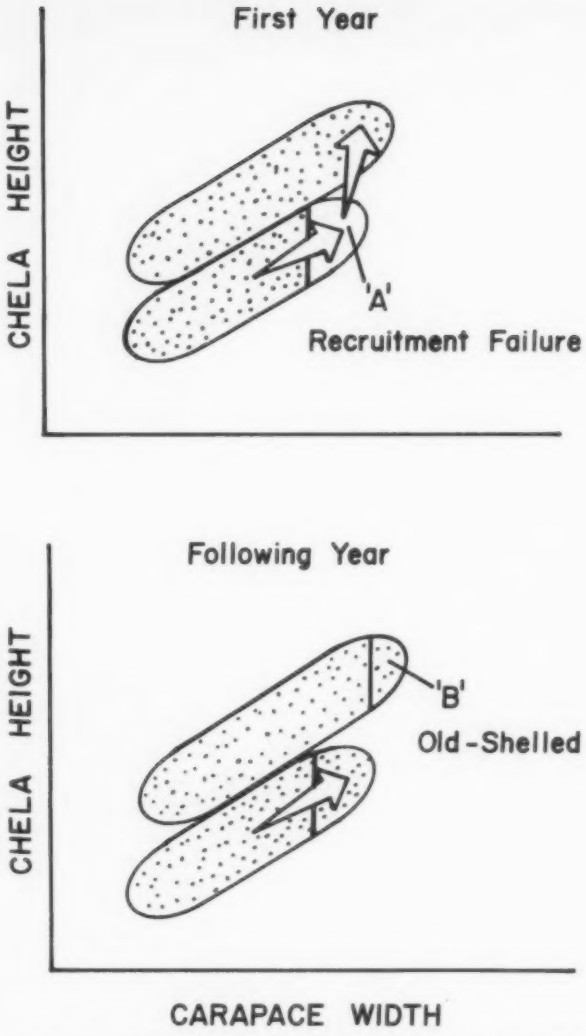

FIG. 1. Hypothetical situation leading to a buildup of old-shelled *C. opilio* males at the largest body sizes. Arrows show the fates of molting animals. Region "A" in the top part of the figure represents animals which are "missing" due to a recruitment failure some time in the past. These animals would normally molt to region "B" shown in the lower part of the figure. In the absence of these recruits, the animals in region "B" all age to the old-shelled condition.

Mouth Parts and Formation of New Carapace as Indicators of Molt Status

In order for a crab to molt, the new (second) carapace must be well formed. Such a new carapace should not occur in large-clawed animals if there is a terminal molt. Dawe et al. (1990) reported finding two males from the Bering Sea with large claws, according to Somerton's (1981) allometric relationship for *C. opilio* (Fig. 2), which clearly had well-developed second carapaces indicating imminent molting. More recently, on June 20, 1990, we found that 26 of a sample of 75 male Bering Sea *C. opilio* landed at Kodiak, Alaska (35%), had well-developed second carapaces. All of those with second carapaces appeared to be large-clawed (Fig. 2). Ito (1970) showed that for large *C. opilio*, sampled by bottom trawl in the Japan Sea, peak occurrence of second carapaces preceded peak occurrence of newly molted (soft-shelled) crabs by 1–2 mo. Thus, examination for second carapaces can provide a powerful and direct method for quantifying molting activity. Unfortunately, examination of specimens for development of a second carapace has not been routinely conducted. Hence, further study will be necessary to determine molting prevalences using this method.

Mouth part morphology has been used to determine the molt status (i.e. postmolt, intermolt, and premolt stages) of snow crab (Moriyasu and Mallet 1986; O'Halloran and O'Dor 1988). Animals in premolt stages D_1 to D_3 are said to be clearly committed to molting. Hence, these stages would not be expected to occur in large-clawed animals if terminal molt exists. There is also a D_0 stage, the beginning of the premolt stage, but in our experience, using the method of Moriyasu and Mallet, this stage is very difficult to separate from the C or intermolt stage.

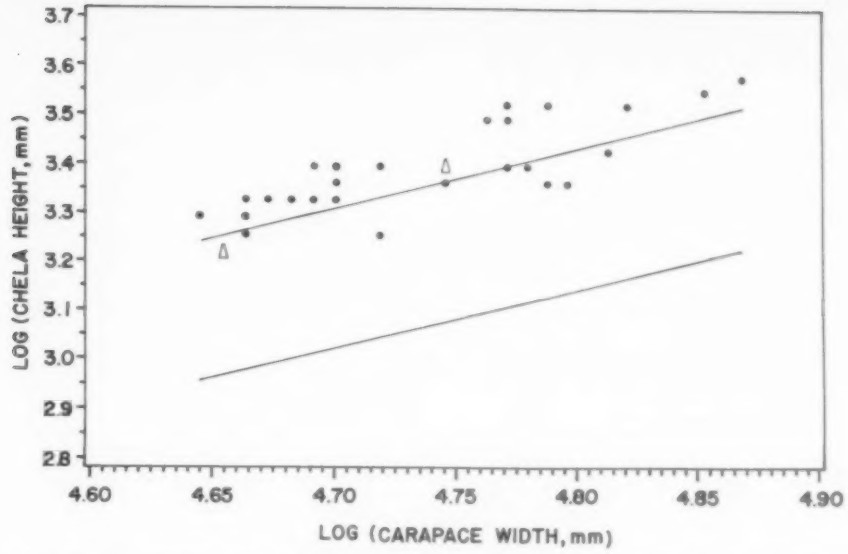

FIG. 2. Relationship between the logarithm of the chela height and the logarithm of the carapace width for Bering Sea *C. opilio* with well-developed second carapaces indicating imminent molting. Open triangles are from Dawe et al. (1990); closed circles are from the current study. The two lines, from Somerton (1981), indicate the central tendencies for clouds of large- and small-clawed animals.

TABLE 1. Samples of trawl-caught male snow crab collected from Conception Bay, Newfoundland, for examination of mouth parts.

(a) Sampling times and sample sizes

Time	Sample size, large-clawed males	Sample size, small-clawed males	Large-clawed molters	Small-clawed molters
March 3, 1988	14	38	1	4
April 25 – May 5, 1988	216	168	0	27
May 12–23, 1989	819	315	1	15

(b) Information on large-clawed males in active premolt

Date captured	Carapace width (mm)	Chela height (mm)	Chela width (mm)	Shell condition	Mouthpart stage
March 1988	60	12.4	10.2	Hard-shell	D_1
May 1989	74	16.2	14.1	Hard-shell	D_1

The D_0 stage is further divided into early D_0 and late D_0, and the early D_0 stage is especially difficult to discern from the C stage. Conan et al. (1988a, p. 1502) also noted the difficulty in identifying premolt stage D_0. Hence, we do not feel that the presence or absence of males in the D_0 stage can be used to address the question of whether or not there is a terminal molt.

We examined the mouth parts of 1049 hard-shelled, clearly large-clawed animals (molt status C and D) greater in size than 60 mm cw and collected by trawl in Conception Bay, Newfoundland, from March to May 1988 and May 1989 (Table 1a). Our defintion of "clearly large-clawed" is any animal above the upper dividing line (based on 1987 data) in Fig. 3. We assume that the timing of our sampling was during the molting season which appears to occur from March to May–June in Conception Bay (Miller and O'Keefe 1981; Dawe et al. 1990). However, the timing of the peak of the molting season appears to be poorly known.

Of the 1049 hard-shelled, large-clawed animals examined in 1988–89, two were clearly committed to molting (Table 1b). Inspection of the relationshp between chela height and carapace width leaves little doubt that these animals were large-clawed (Fig. 3). We also examined 521 clearly small-clawed animals in molt stages C to D (i.e. with hard carapaces) which had a similar size composition as the large-clawed animals (mean size of small-clawed animals = 73 mm with standard deviation = 9.0 mm; mean size of large-clawed animals = 76 mm with standard deviation = 13.5 mm). Of the small-clawed males, 46 (9%) were in D_1 or above. Clearly, we did not find a large percentage of either large- or small-clawed males in active premolt. However, it is notable that the sampling in the first part of the molting season (March) was extremely small (38 small-clawed and 14 large-clawed).

Conan and Comeau (1986) examined the mouth parts from 260 large-clawed males caught in trawls from July to December, and also from 110 males (presumably large-clawed) taken in traps at an unspecified time of year. The size distribution and shell conditions of the animals were not given. They stated that none of the individuals was "in a premolt "D" or molt "A" stage." However, stage "A" (postmolt) sheds no light on the terminal molt question, since even animals molting to a terminal molt condition would undergo a postmolt stage. Conan and Comeau concluded that an upper, one-sided, 95% confidence limit on the proportion that ever molt is 0.8%, i.e. virtually nil. However, this analysis suffers from several flaws:

(1) Some animals may not molt every year. Also, not all the animals that will molt during the year will be in premolt stage at the exact same time. Therefore, Conan and Comeau's procedure estimates the proportion of animals molting at the time of sampling, rather than during the whole year or during any subsequent period of life.

(2) The molting season for male snow crab appears to be in the late winter – spring (Miller and O'Keefe 1981; Conan et al. 1988a, 1988b); thus, Conan and Comeau looked for initiation

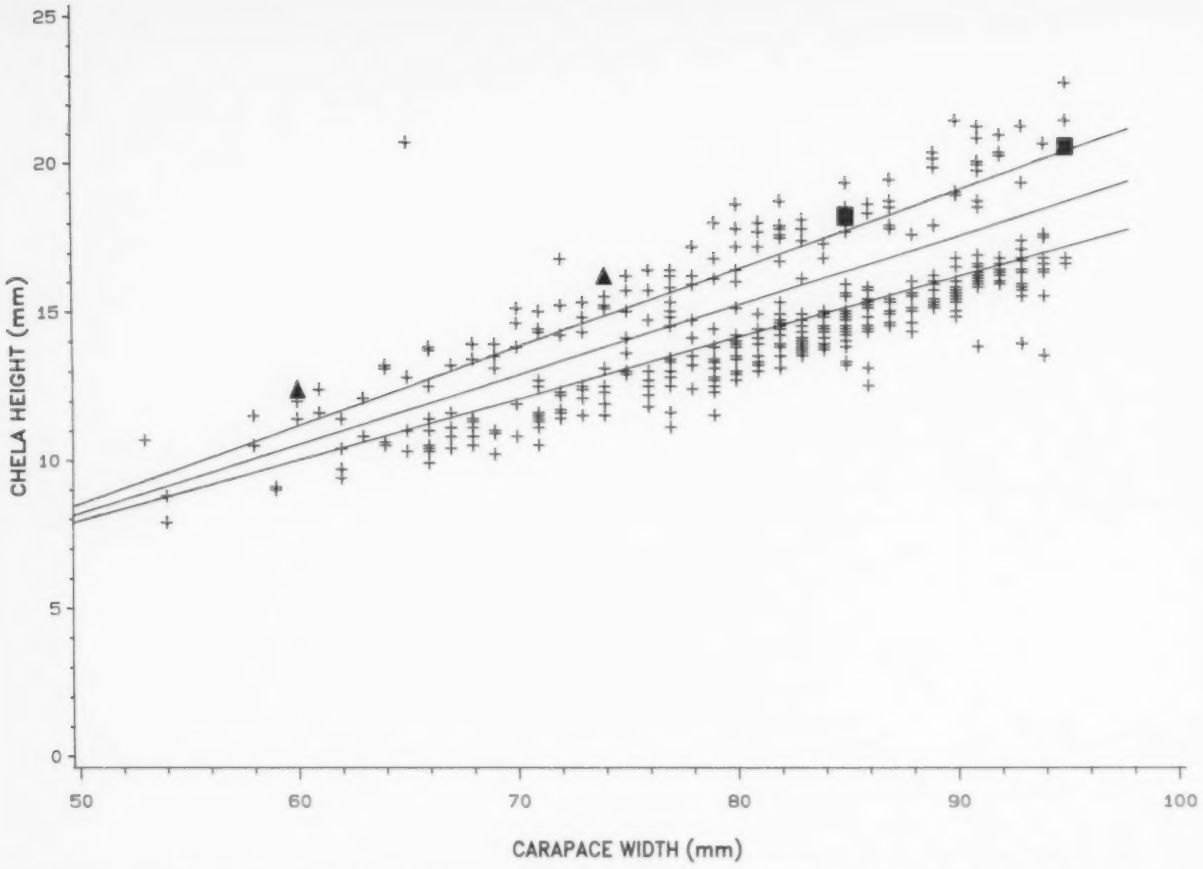

FIG. 3. Relationship between chela height and carapace width for a sample of 400 male *C. opilio* collected from Conception Bay, Newfoundland, in 1987 by trapping. The middle line appears to separate well the clouds of large-clawed and small-clawed animals. We refer to animals above the upper line as "clearly large-clawed" and those below the lower line as "clearly small-clawed." Lines were drawn by eye. Triangles refer to two animals with mouth parts indicating the D_1 phase of the molt cycle (i.e. clearly committed to molting). Squares refer to the morphometry at time of tagging of two animals which subsequently molted.

of molting and active molting after the molting season was largely over.

(3) The proper computation is not what proportion of the large-clawed animals are molting or about to molt; rather, it is what proportion of the animals that could potentially molt (i.e. are hard-shelled) are molting or about to molt; such information was not provided.

(4) If animals about to molt do not feed, as reported by O'Halloran and O'Dor (1988) for aquarium-held animals which molted, then the animals caught in baited traps would not be expected to show signs of molting activity, and trapped animals should not be used to study molting activity as was done by Conan and Comeau.

(5) Conan and Comeau reported that "premolt and molting immatures are frequently found in trawl catches." However, they did not specifically report the number or proportion of animals with small claws that were molting in the catches from which they obtained the large-clawed animals. The absence of large-clawed animals in a molting condition is certainly not meaningful if molting small-clawed animals are also missing.

For these reasons, the mouth part data of Conan and Comeau (1986) do not appear to shed light on the question of terminal molt.

Conan et al. (1988a) addressed some of the above concerns. Concerning points 2 and 5 (seasonal timing); they reported that no "premolt" animals were found among 6081 mature (i.e. large-clawed) animals and 2004 immature (i.e. small-clawed) animals caught by trawl from July to September 1987. (They did not consider trapped animals, since (pre)molting animals would not be susceptible to capture in baited traps (point 4).) From October to November (year not specified), they obtained the following results from trawling:

Group	Number examined	Number in premolt stage
Large-clawed	312	0
Small-clawed	208	50 in D_0

Note that no animals, large- or small-clawed, were found in active premolt stages D_1 to D_3. Thus, even among the small-clawed animals, they did not find animals clearly committed to molting at the times they sampled. Animals in late D_0 molt in 8–12 wk whereas animals can remain in early D_0 for several months according to O'Halloran and O'Dor's (1988) findings

for laboratory animals. If Conan et al.'s males were in late D_0, most would apparently molt well before the molting season; therefore, most were presumably in early D_0. As explained above, we find that the distinction between the intermolt stage C and the beginning of premolt, especially early stage D_0, is dubious. Therefore, we feel that there was little evidence that either group of animals showed signs of imminent molting activity. Also, Conan et al. did not provide a breakdown by shell condition, mouth part stage, or body size of the animals they examined. Some of the 520 animals they examined may have been soft-shelled (including crabs with brittle carapaces whose chelae are easily shattered by thumb pressure). If so, then these animals could not be expected to be preparing for another molt and should not be used in a comparison of prevalence of molting among large- and small-clawed animals. The distribution of body size may have differed between the two groups of animals. In this case, comparisons of prevalence of molting would again not be appropriate.

Conan et al. (1990) reported on additional observations of crab mouth parts. Their report suffers from the same deficiencies of their earlier papers: lack of detail concerning the specimens examined, sampling at an inappropriate time of the year, and apparent heavy reliance on stage D_0 upon which to base interpretations. (They reported results for stages D_0 and D_1 combined; however, the D_0 stage is unreliable in our opinion, and they sampled from October to November, i.e. 3–6 mo before molting, when they should have encountered very few animals in D_1.)

Origin of the Largest of the Large-Clawed Males

Consider the scatter plot of trap-caught animals from Conception Bay, Newfoundland, in Fig. 4. The largest of the small-clawed animals was 102 mm cw except for a single animal of 116 mm cw. When animals of this size molt, they increase in size about 12 mm cw according to results from tagging studies carried out in Conception Bay (Taylor and Hoenig 1990). Thus, the animals would be expected to be about 114–117 mm cw when they reach their terminal molt, if terminal molt exists. The largest of the large-clawed animals was 134 mm cw, i.e., at least 20–30 mm larger than the largest small-clawed animals would be after they undergo their terminal molt. This suggests strongly that some of the large-clawed animals must be molting in order to account for the occurrence of the very large, large-clawed animals.

The gap between the largest of the small-clawed males and the largest of the large-clawed males in Conception Bay is about 32 mm. Similar diagrams have been produced for a wide variety of locations and years (Dawe et al. 1990). However, interpretation of such data depends critically on the availability of reliable estimates of growth increments (see below).

Conan and Comeau (1986) stated that "The size difference between larger morphometrically immature males and larger morphometrically mature ones in our data is 120–130 mm L_c (Fig. 4b) and can easily be covered over one molt to maturity." Examination of that figure reveals, however, that the difference is actually about 19 mm as opposed to 10 mm. In a later paper, Conan et al. (1990) acknowledged that gaps larger than 30 mm occur and stated "A size increment at molt of 30 mm is well within the range of possibility for a 120 mm male. Miller and Watson's (1976) data suggest average growth increments of 20% or more." A 20% increment in size is not sufficient to account for the observed gaps in the scatter plots discussed by Conan et al. (1990). Furthermore, Miller and Watson reported an average growth increment of 18.4% for captive animals larger than 57 mm (and larger average percentage increments only in size categories smaller than 57 mm). The greatest individual molt increment observed in their data on large (57–100 mm cw) experimental males was about 22%. Even if all males were capable of such large increments, this would result in a size increment of only 26 mm for a 120 mm cw male, i.e. not large enough to account for the observed gaps.

The available estimates of growth increments are quite variable, as shown in the table below:

		Average increment at 120 mm		
Reference	Method	Source	mm	%
Taylor and Hoenig 1990	Tagging	Conception Bay	12	10.0
Moriyasu et al. 1987	Aquarium		16	13.3
Miller and Watson 1976	Aquarium		22	18.4
Comeau et al. 1989	Modal analysis	Bonne Bay	30	25.0
G. P. Ennis, unpubl. obs.	Field/aquarium	Bonne Bay	29	24.1

The only estimates higher than those reported by Miller and Watson are for Bonne Bay, Newfoundland, and these are for animals considerably smaller than 120 mm. We estimated increments for Bonne Bay animals at 120 mm cw based on the assumption that the percentage increase is the same as for small animals. For this reason, the estimates are probably too high. Also, the estimate from Comeau et al. (1989) pertains to molting animals which retained the small-claw state. Comeau et al. (1989) reported that animals molting to the large-claw state have smaller increments which "agree with the size increment presented by Miller and Watson (1976) for larger crabs." Thus, available growth estimates do not account for the gaps in sizes observed in nature.

The large gap in size between the largest small-clawed animals and the largest large-clawed animals could also be explained by recruitment failure in the past, which could result in the absence of a given size group of small-clawed males. This appears to have been the case in Bonne Bay, Newfoundland, in the 1980s. Comeau et al. (1989) and Comeau et al. (1991) showed that the maximum size of the small-clawed males in Bonne Bay increased steadily over a period of several years. However, gaps within size–frequency distributions (which would also be evident if recruitment failed) are rarely encountered. Furthermore, it is unlikely that recruitment failure could account for the missing largest small-clawed animals in all the scatter plots examined (Dawe et al. 1990).

Another possibility suggested by Conan and Comeau (1986) is that the wide gap in body size between the largest of the small-clawed animals and the largest of the large-clawed animals, reported by others, is "most likely a sampling artifact" and explain that "some types of gear such as traps or trawls which do not rake the sediment efficiently enough do not capture large morphometrically immature males which tend to conceal themselves more than mature ones, as shown by the aquarium experiments." The aquarium evidence to which Conan and Comeau referred suggests only that under crowded, artificial

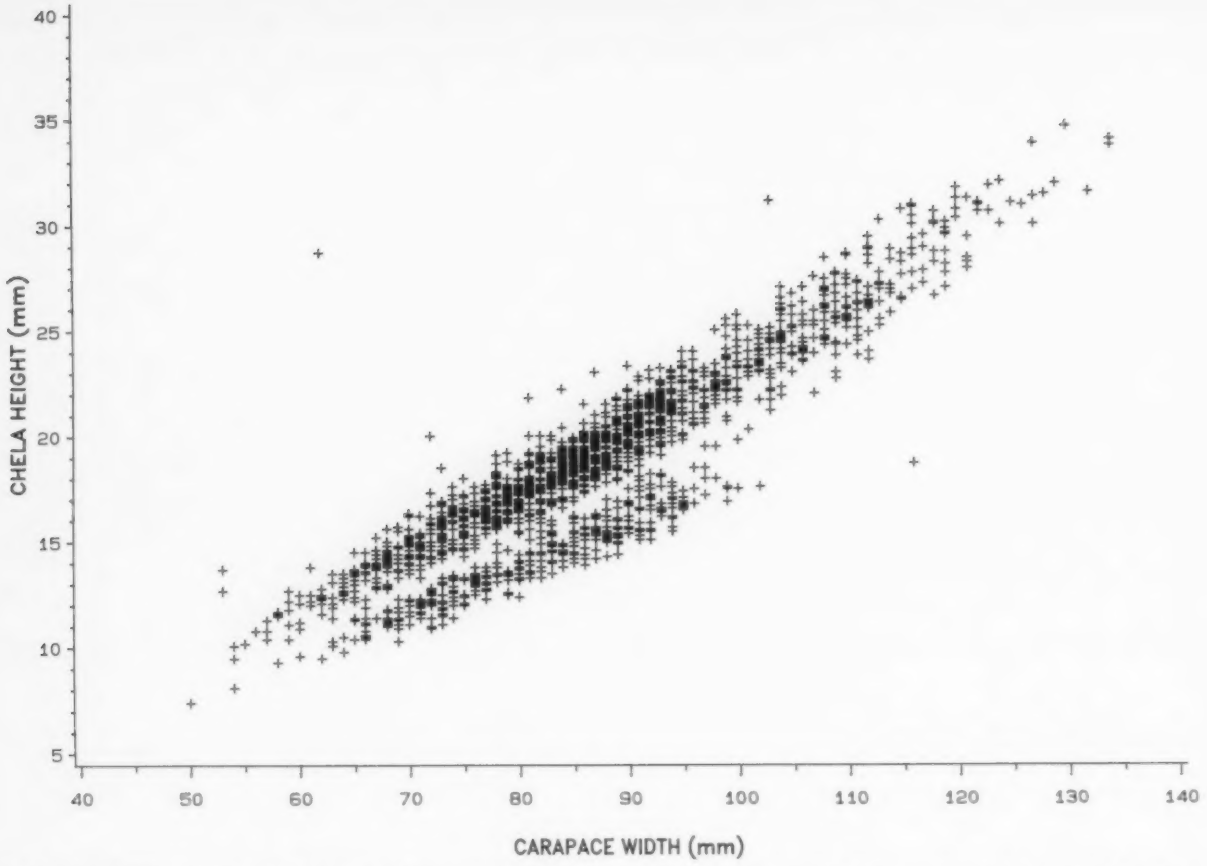

FIG. 4. Relationshp between chela height and carapace width for male *C. opilio* from Conception Bay, Newfoundland, captured in traps in September–October 1989.

conditions, when males are competing for females, the small-clawed males may not be as aggressive or successful as large-clawed males with larger body sizes and may exhibit a "hiding behavior" by placing stones on their backs.

Conan et al. (1990) advanced one more explanation for the size gap between the largest of the small-clawed males and the largest of the large-clawed males. They suggested that the body size at which males make the transition in claw size may vary from year to year. In some years, the animals may develop large claws at a large body size, thus giving rise to extremely large, large-clawed males. In a subsequent year, the transition may be made when the males have a small body size, thus eliminating the largest of the small-clawed males from the population and widening the size gap. This is a new and speculative hypothesis concerning size-synchronized molting and the effects of annual variability. The only supporting evidence given by Conan et al. is that, in the Gulf of St. Lawrence, the magnitude of the gap has differed according to three field surveys conducted at widely separated times. A mixture of sampling gears was used in these studies, so the results are not interpretable. Another study which reported yearly changes in the magnitude of such gaps was described by Comeau et al. (1991) who showed that the maximum size of small-clawed males and the minimum size of large-clawed males in Bonne Bay changed over a period of several years in the 1980s. They interpreted these changes as a response to changing crab densities. In our opinion, these observations do not provide evidence for Conan et al.'s (1990) proposed annual variability in the size at transition to the large-claw state.

TABLE 2. Comparison of times at liberty for recaptured animals tagged in large-clawed versus small-clawed condition in May 1988 in Conception Bay, Newfoundland.

Time at liberty (mo)	Large-clawed (no. of recaptures (%))	Small-clawed (no. of recaptures (%))
4–5	33 (49)	4 (21)
13–17	34 (51)	15 (79)

Growth of Tagged Animals

A tagging study was initiated in 1987 in Conception Bay, Newfoundland, with one of the goals to determine if large-clawed animals molt. The relationship between chela height and carapace width was examined for 400 animals collected from Conception Bay in 1987 (Fig. 3). Only animals which clearly lay above the upper line or below the lower line in Fig. 3 were tagged in 1988 and 1989. T-bar tags were applied in the

TABLE 3. Tag and recapture data for two "clearly large-clawed" animals from Conception Bay, Newfoundland, which molted while at liberty.

Characteristic	Animal 1	Animal 2
Tag No.	G2138	G3172
Date tagged	May 14, 1988	May 11, 1988
Carapace width at tagging (mm)	85	95
Chela width at tagging (mm)	16.3	17.8
Chela height at tagging (mm)	18.2	20.5
Shell condition at tagging	New-hard-shelled	New-hard-shelled
Date recaptured	Sept. 8, 1989	Sept. 8, 1988
Time at liberty (d)	482	120
Carapace width at recapture (mm)	100	103
Change in size while at liberty (mm)	15 (18%)	8 (8%)
Chela width at recapture (mm)	?	Unavailable[a]
Chela height at recapture (mm)	23.1	Unavailable[a]
Shell condition at recapture	New-hard-shelled	New-hard-shelled

[a]Specimen was returned without claws by fisherman.

ecdysial suture according to methods described by Taylor and Hoenig (1990). To date, 67 large-clawed and 19 small-clawed tagged animals have been recaptured from the first experiment in 1988 (Table 2). Two large-clawed animals (Table 3; Fig. 3) and 13 small-clawed animals molted while at liberty. Note that the small-clawed animals recovered were at liberty for longer periods of time, on average, than the large-clawed animals (Table 2), thus providing the small-clawed animals more opportunity to molt than the large-clawed animals.

One of the large-clawed males which molted was at liberty for 120 d (4 mo). It was tagged as a new-hard-shelled (shell condition 2) animal (see Miller and O'Keefe 1981 for definitions of shell conditions) and recovered as a new-hard-shelled animal. The 4 mo at liberty were sufficient for the crab to fast for a month, molt, and recover to a hard-shelled condition before recapture (Taylor et al. 1989). This presumes that molting took place in June which, as already noted, is quite possible for snow crab in Conception Bay. The other large-clawed male recaptured was at liberty for 16 mo and was tagged and recaptured in the new-hard-shelled condition. Clearly, this animal had time to molt and recover to a hard-shelled condition. Dawe et al. (1990) noted that the growth increments of these two large-clawed males were consistent with growth estimates from tagging studies.

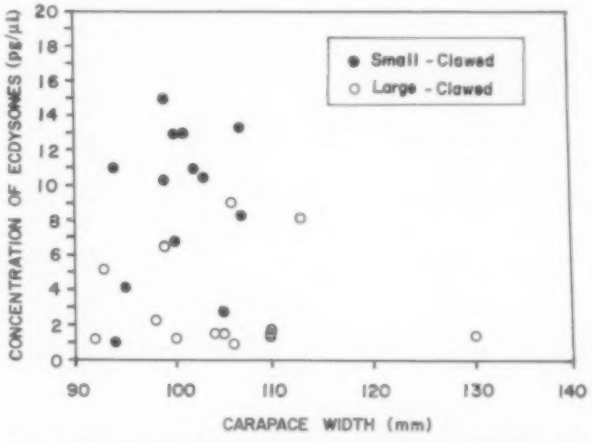

FIG. 5. Concentration of ecdysones versus carapace width for male *C. opilio* (from Cormier 1986, fig. 3.8). Note the considerable overlap in the distributions of hormone concentrations for small-clawed and large-clawed males of similar body sizes.

Other Evidence

Hormones and Y-Organs

Conan et al. (1988a, 1988b) suggested that levels of the molting hormone crustecdysone are low and the Y-organs are reduced in large-clawed animals. They presented no new information but, rather, cited work by Cormier (1986) for the level of crustecdysone. They gave no reference for Y-organs, and the only reference of which we are aware (Rioux 1988) is about females. E. Bataller and A. Boghen are studying morphology of the Y-organ in males and report the following (A. Boghen, Université de Moncton, April 9 and 26, 1991, pers. comm.): there is a full or partial transformation of the Y-organ sometime after the male snow crab attains large-claw status and this transformation seems to correspond to the degenerative process described for other species; however, the results are not definitive although they are consistent with the idea of a terminal molt. They believe that the Y-organ continues to show some activity for an unknown period of time after the molt to the large-clawed state.

Levels of crustecdysone, and condition of the Y-organ, are probably related to body size of the crab, position in the molt cycle (as evidenced by shell condition), and season of the year. Comparisons of levels of molting hormone and condition of the Y-organ between small- and large-clawed males can only be done after accounting for these factors, as suggested by Conan et al. (1988a) for crustecdysone. Figure 5 (from Cormier 1986, fig. 3.8) suggests that over the size range from 92 to 114 mm cw, small-clawed males have higher levels of crustecdysone on average than comparably sized large-clawed males. However, there is a great deal of overlap of the distribution which needs explanation. It is not clear what level of crustecdysone is necessary for molting to occur and how the levels change over the course of the molting cycle.

It is possible to establish experimentally the physiological ability of a crab to molt. For example, Havens and McConaugha (1990) questioned the conventional wisdom that female blue

crab (*Callinectes sapidus*) undergo a terminal molt. As part of their study, they ablated the eyestalks of captive animals to eliminate the production of molt-inhibiting hormone. They concluded that mature females were physiologically capable of molting (the Y-organ continues to produce the molting hormone), since their experimental animals all molted (except three which died shortly after the ablation.)

Limb Regeneration

Conan et al. (1988b, p. 48) stated that large-clawed males never regenerate limbs, while small-clawed males do. They cited no references and provided no data. Consequently, there is no way to evaluate this. We note that the absence of regenerating limb buds in large-clawed males could only be considered significant if small-clawed crabs of the same size have regenerating limbs. Conan et al. (1990) reported that they found no reference to any experiment showing that limbs of postpubberal (i.e. large-clawed) majids can regenerate, but they did not report finding any experiments showing the contrary, either. It would be possible to bring similarly sized large- and small-clawed males into the laboratory, remove a limb, and look for signs of limb regeneration.

Evidence from Similar Species

In the original paper on terminal molt of snow crabs, Conan and Comeau (1986) noted that, with the exception of the genus *Chionoecetes*, male crabs in the family Majidae are generally accepted to have a terminal molt. They then suggested that their work on *C. opilio* and their review of the literature on other species of *Chionoecetes* were sufficient to conclude that males of all species in the family Majidae undergo a terminal molt. This view was challenged by Donaldson and Johnson (1988) and Donaldson (1988). In response to Donaldson and Johnson, Conan et al. (1988a) conceded that terminal molt of males may not exist in all species of the genus *Chionoecetes* and, in referring to the rather minor degree of differentiation in claw size occurring in *Chionoecetes* (relative to that in other majids), stated that "This may suggest that *Chionoecetes* have not yet fully made the choice for a terminal molt as other genera of majids." They further acknowledged that some populations of *C. opilio* may undergo a terminal molt, while others do not. However, in Conan et al. (1990), it is again suggested that terminal molt occurs throughout the genus without providing any new evidence for the other species of *Chionoecetes*. That conclusion is not consistent with the observation of Donaldson and Johnson that large-clawed male *C. bairdi* molted in holding pens.

If the evidence were clear that males of all species in the Majidae undergo a terminal molt except *C. opilio*, then one would be well advised to consider carefully if *C. opilio* is really different from all other species in the family. However, universality of terminal molt within the family is not accepted to be the case. Therefore, circumstantial evidence from other species of majids does not provide strong evidence for (or against) terminal molt in *C. opilio* males.

Conclusions

We conclude from our review of the available literature that the existence of a terminal molt, or a greatly reduced molting frequency, in large-clawed or large-bodied or old male snow crab (*Chionoecetes opilio*) is probable but not well supported by the data or the analyses. We have presented direct evidence, albeit limited, which is not consistent with the idea of a terminal molt in males of this species. Further research is required to resolve the question, but clearly the conclusion that a terminal molt exists in male snow crab is untenable at this point.

We have tried to stress that the quantitative assessment of the prevalence of molting of large-clawed males is possible but that it is difficult because timing of the molting season is poorly known and because body size and molting history (as indicated by shell condition) are factors which affect the molting of individuals. These factors must be accounted for if a valid quantitative comparison of molting prevalences is to be made between large-clawed and small-clawed male snow crab.

Acknowledgments

We thank Scott Akenhead, Don Parsons, and Michel Comeau for comments on an earlier draft and Eric Bataller and Andrew Boghen for sharing unpublished results with us. David Somerton graciously provided source material from his 1981 paper on tanner crab. Paul Collins and Noel Cadigan helped with the graphics.

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Interactions with the Benthos Alter Pelagic Food Web Structure in Coastal Waters

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Results from studies in experimental enclosures containing both water column and benthic communities show that the benthos has an important effect on the structure and productivity of pelagic food webs whether the system is nutrient enriched or nutrient limited. Research over a 10-yr period in 13-m^3 mesocosms showed that changes in the pelagic food web were correlated with different sediment communities and with the presence or absence of a benthos. The abundance of copepods was inversely correlated with numbers of macrofauna. At both low and high nutrient levels, systems without benthos had greatly enhanced numbers of carnivorous holozooplankton including ctenophores, medusans, chaetognaths, and fish. Our observations indicate that the presence of the benthos shortens the pelagic food web and inhibits the response of pelagic fauna to nutrient enrichment in well-mixed coastal waters. The strength of benthic–pelagic coupling, which is controlled by the amount of turbulence in the water column, may be more important to food web structure than the rate of nutrient supply and could determine which subsystem responds to eutrophication.

Des études en enclos expérimentaux contenant à la fois la colonne d'eau et le benthos révèlent l'effet considérable de celui-ci sur la structure et la productivité des réseaux alimentaires pélagiques, que le système soit enrichi ou carencé en matières organiques. Des travaux étalés sur 10 ans dans des mésocosmes de 13 m^3 révèlent que les changements dans le réseau alimentaire sont liés aux diverses biocénoses des sédiments et à la présence ou à l'absence du benthos. L'abondance des copépodes était inversement corrélée à celle de la macrofaune. À des concentrations faible et élevée de matières nutritives, les biocénoses sans benthos présentaient beaucoup plus de carnivores holoplanctoniques (cténophores, méduses, chétognates, poissons). Nos observations indiquent que la présence du benthos raccourcit la chaîne alimentaire pélagique et inhibe la réaction de la faune pélagique à l'enrichissement en matières nutritives des eaux côtières bien mélangées. La force du couplage benthos–pélagos, régie par la quantité de turbulence dans la colonne d'eau, pourrait influer plus sur la structure du réseau alimentaire que le taux d'apport en matières nutritives et pourrait déterminer quel système réagit à l'eutrophisation.

Received November 28, 1990
Accepted June 4, 1991
(JA827)

Reçu le 28 novembre 1990
Accepté le 4 juin 1991

In shallow seas, bays, and estuaries both benthic and pelagic communities play an important role in ecosystem function (Odum 1967). Much of the organic matter entering the benthic food web derives from the plankton. In turn, the benthos returns inorganic plant nutrients to the overlying water. The importance and magnitude of this biogeochemical interaction between pelagic and benthic subsystems have been well established (Hargrave 1973; Zeitzschel 1980; Kelly and Nixon 1984); however, the biotic interactions between the two communities have not been completely explored. When interactions between benthos and water column are examined, it is the flow of phytoplankton to benthic herbivores which is most often considered, while benthic influence on phytoplankton, and especially zooplankton dynamics, has received less attention.

Marine ecologists have only recently begun to realize that benthic communities can significantly affect the composition and abundance of pelagic populations. The potential control of algal populations by bottom organisms has been suggested (e.g. Kremer and Nixon 1978; Dame et al. 1980; Cloern 1982; Officer et al. 1982; Herman and Scholten 1990) but no demonstrative experimental evidence exists to test these hypotheses. Initial attention has focused on filter-feeding bivalves because of their direct effect on phytoplankton biomass (Doering et al. 1986). Such organisms can alter phytoplankton, and possibly zooplankton, abundance on time scales equivalent to the tidal cycle (Carlson et al. 1984). Longer term effects such as suppression of phytoplankton blooms and alteration of pelagic food web structure have been hypothesized (Nichols 1985). Observations of the size structure of benthic and planktonic communities led Warwick and Joint (1987) and Schwinghamer (1985) to hypothesize that, in shallow waters, benthic–pelagic coupling leads to trophic interactions affecting pelagic community structure, especially in the size range of macrozooplankton. Daborn (1986) noted that where macrofauna are abundant, zooplankton are not, implying a suppression of zooplankton by the benthos in shallow, tidally mixed systems.

Officer et al. (1982) postulated criteria for natural systems in which benthic control is likely to be important, including high nutrient supply, shallow water depths, and an extensive benthic filter-feeding population (bivalves). These characteristics limit the general relevance to a few shallow coastal embayments. However, we have repeatedly observed apparent benthic control of pelagic food webs during a 10-yr series of experiments in 13-m^3 enclosures with a variety of intact benthic communities and at low and high rates of nutrient supply. Benthic grazing and predation appear to have an even greater influence over pelagic productivity, and especially food web structure, than does the biogeochemical action (nutrient recy-

cling) of the benthos (such as described in Kelly et al. 1985). This effect is independent of the rate of nutrient supply to the system. We have come to these conclusions and some understanding of the mechanisms involved by reviewing our results on zooplankton community structure from experiments during 1979–86. We present here a synthesis of data describing the effects of benthic control over planktonic food web structure and productivity in both nutrient-limited and nutrient-enriched systems used to model shallow coastal areas.

Much of our information was derived from experiments on nutrient effects in estuaries. With each experiment we became more convinced that a complete understanding of nutrient limitation and eutrophication required a better knowledge of biotic interactions between the benthos and water column. There are some important implications of these results that warrant consideration. For instance, in coastal systems where the benthos controls primary production, the response to eutrophication will be quite different from areas where it does not. Activities which significantly alter benthic community composition will be reflected in the water column community and may have important impacts on water quality. In addition, if competition between benthos and water column species for food resources affects pelagic community structure, the degree of benthic–pelagic coupling will alter the balance between production of pelagic versus demersal fisheries, as suggested by Cloern (1982) and others (such as Townsend and Cammen 1988). We suggest that an improved knowledge of the factors which determine the strength of benthic–pelagic interactions is essential to a complete understanding of dynamics of coastal ecosystems. Those physical, chemical, and geological factors, as well as the effects of species composition of the benthos (potentially controlled by man's activities), which are involved in linking water column and benthos need to be more thoroughly explored in both field and laboratory experiments.

Experiments at the Marine Ecosystems Research Laboratory (1979–85) conducted in 13-m^3 enclosures with a benthos indicated an inverse relationship between standing stocks of benthic macrofauna or their larvae, and holozooplankton. These statistical correlations implied the existence of competitive and/or predatory interactions which enable the benthos to use newly produced carbon more readily than zooplankton. Here we review correlative data from enclosure studies with benthos and summarize results of an ecosystem level manipulation (1985–86) which directly tested the hypothesis that the benthic community influences pelagic food web structure in shallow coastal ecosystems.

Methods

Methods used in these experiments have previously been published (Oviatt et al. 1948, 1987; Sullivan and Ritacco 1985; Doering et al. 1989) but relevant details are reviewed here. The primary focus here is on three experiments: (1) August 1979 to April 1981, sediment gradient experiment; (2) June 1981 to October 1983, nutrient gradient experiment, and (3) June 1985 to June 1986, sediment by nutrient interaction (Table 1). During all experiments, flushing and mixing were the same. New seawater flowed to the tanks every 6 h at the approximate rate of 120 L over a 12-min period providing an exchange of 3.7%·d^{-1} and a turnover time of 27 d which is similar to the mean flushing time of Narragansett Bay (Pilson 1985a). Mixing was designed to simulate the intensity of tidal currents and occurred on a schedule of 2 h on and 4 h off. Mixing was accomplished by the vertical rotation (4–5 rpm) of a plunger located about 0.6 m above the sediment. The scaling criterion for mixing vigor was to resuspend bottom sediments to the same levels (3–5 mg·L^{-1}) that are observed in Narragansett Bay. Sediments in all three experiments were taken by a spade corer (0.25 $m^2 \times 0.37$ m) from areas in or near Narragansett Bay. In the first experiment, sediments predominating in silt–clay fractions were obtained in August from three locations in the Narrangansett Bay region (Table 1). In the second experiment, all mesocosms contained silt–clay sediments obtained in May from mid-Narragansett Bay. In the final experiment, sediments were obtained in May from the same location and placed in half the mesocosms in each treatment; the other mesocosms received no sediments. The 1981–83 experiment included six nutrient addition treatments that were geometric multiples (1× up to 32×) of 2.88 mmol N·m^{-2}·d^{-1}, 0.22 mmol P·m^{-2}·d^{-1}, and 0.21 mmol Si·m^{-2}·d^{-1}. Three other mesocosms with no nutrients served as controls for this gradient experiment. The 1985–86 experiment included replicate control and nutrient-enriched mesocosms both with and without sediments and an intact benthic community and were sampled for 1 yr. The design was two control mesocosms with sediment, two control mesocosms without sediment, two nutrient treatments with sediment, and two nutrient treatments without sediment. Nutrient treatment was identical to that at the 8× level in the 1981–83 experiment. By omitting the benthos while allowing floc to settle out of the water column and accumulate, benthic macrofauna were eliminated but the biogeochemical role of the sediments was mostly retained. While there were some differences in benthic nutrient fluxes between sediment and no-sediment treatments, it was evident that presence versus absence of the macrofauna or sediments itself was the treatment effect most responsible for the differences observed (Doering et al. 1989).

Methods used to estimate biological parameters were the same for all exper.nents. Biomass of phytoplankton was determined weekly by analysis of chlorophyll *a* after Yentsch and Menzel (1963) and Lorenzen (1966) as modified by Lambert and Oviatt (1986). Diatom counts were obtained from a pooled sample from three depths taken weekly during bloom periods and less frequently during periods of low concentration. Samples were preserved in Lugol's iodine solution and placed in a settling chamber prior to counting. The sample was allowed to settle for 20 h and then counted with an inverted microscope. A known area of the chamber was counted until at least 500 cells or colonies of the three most numerous species were counted. Each 100 cells or colonies counted for a species resulted in a precision of 10%.

Zooplankton abundances and species composition were estimated from weekly net tows (two replicates) in summer, every 2 wk in winter, sampling from bottom to top of each tank. Nets were 30 cm in diameter, 64-μm mesh, and sampled 0.22 m^3 per tow. The net mesh was 150 μm during 1981–82 only; adult copepods, adequately sampled by both meshes, are therefore used in comparisons across years. Ctenophores were removed with a 1-mm mesh prior to sample preservation and counted live. Subsampling was done with a 2-mL Stempel pipette to produce a count of 200 or more animals. Biomass (>150 μm fraction) was analyzed from a separate tow rinsed with deionized water, frozen, and processed in a freeze-dryer. Weights of samples contaminated with phytoplankton were estimated from a relationship between zooplankton numbers and biomass from uncontaminated tows.

TABLE 1. Description of selected experiments conducted at the Marine Ecosystems Research Laboratory Narragansett, RI, during 1979–85.

	Sediment origin	No. of mesocosms	Treatment
I. August 1979 to April 1981	Providence River	3	Sediment type
	Mid-Narragansett Bay	3	Sediment type
	Rhode Island Sound	3	Sediment type
II. May 1981 to September 1983	Mid-Narragansett Bay	3	Control
	Mid-Narragansett Bay	6	Nutrient addition, 1–32×
III. May 1985 to June 1986	Mid-Narragansett Bay	2	Control
	Mid-Narragansett Bay	2	Nutrient addition, 8×
	No sediment	2	Control
	No sediment	2	Nutrient addition, 8×

Juvenile Atlantic menhaden (*Brevoortia tyrannus*) were sampled every 2 wk by towing a circular net (diameter just smaller than mesocosm diameter). Fish were removed briefly for measurement of total length and live ones replaced in mesocosms. A length – dry weight relationship was determined from any fish that died.

Benthic fauna samples were collected prior to treatment and at least during June, September, and January of each year. During all seasons, additional samples were taken but sometimes not counted. For each sampling, 10 random sediment samples were collected with a flow-through corer (5.067 cm^2) to a depth of 10 cm. The samples were sliced to yield depth fractions of 0–2 and 2–6 cm, and each fraction was preserved in 10% buffered formalin containing rose bengal. After 2 mo (to stabilize stain uptake and weight), the samples were washed through a nest of sieves (0.5 and 0.3 mm) into gridded Petri dishes. Animals were identified and enumerated under a stereomicroscope.

Feeding rates of the mud anemone (*Cerianthiopsis americanus*) were obtained in a 0.35-m^3 fiberglass aquarium mixed vertically with a Plexiglas plunger with variable speed control. Sediment from which other large macrofauna (bivalves, shrimp, amphipods) had been removed was placed in the bottom and a known number of anemones was added. The aquarium was filled with unfiltered overflow water from control mesocosms. Prey density was estimated from replicate 5-L samples withdrawn using a siphon following complete mixing of the water column at the beginning and end of each 24-h experiment. The aquarium was outdoors (natural lighting) and temperature was controlled by immersion in a water bath pumped from Narragansett Bay. Extensive observation of the anemones' feeding behavior both in the aquaria and 2.5-L glass beakers confirmed the conclusion that vertical stirring accelerated feeding rates (i.e. plankton mortality was due to ingestion, not the mechanical effect of the plunger).

Results

The initial stimulus for our thinking about benthic–pelagic interactions came from the system response to changes in methods of sediment collection. During the 1979–80 sediment–gradient experiment, numbers of benthic macrofauna were quite low (Table 2). The 1979 sediment collection resulted in high benthic mortality in sediments due to uncontrolled temperature during recovery. The development of large numbers of the shrimp *Crangon septemspinosa* prevented subsequent development of macrofauna in these mesocosms (Oviatt et al. 1984). Correlating with these low numbers of benthic macrofauna were high numbers of copepods together with a common water column predator, the ctenophore *Mnemiopsis leidyi* (Table 2). Abundance of these planktonic groups was lower in control mesocosms from other experiments where macrobenthic numbers were higher. Thus, zooplankton abundance and species composition in the mesocosms were related to benthic abundance in this rather special case. However, results from the 1981–83 nutrient gradient experiment were also indicative of inverse correlations between benthic and planktonic fauna. While a highly significant semi-log correlation between primary production in the water column and nutrient loading was observed in nine enclosures with sediments (Oviatt et al. 1986), the response of the benthic and zooplankton communities varied from year to year and was not easily related to nutrient input (Fig. 1). During 1981–82, abundance of benthic fauna correlated with nutrient input due to an increase in the abundance of surface suspension feeders which predominated the macrofauna (Fig. 1a, top panel); numbers of benthic larvae (meroplankton) in the water column also increased with nutrient supply (Fig. 1a, bottom panel), reflecting increased rates of reproduction by polychaetes (Levin 1986). In contrast, numbers of holozooplankton (primarily copepods) were unaffected by nutrient treatment (Fig. 1a, top panel) despite increases in individual rates of egg production (Sullivan and Ritacco 1985). The benthos in those mesocosms with low zooplankton numbers was characterized by very dense populations of the suspension-feeding bivalve *Mulinia lateralis* and the capitellid and spionid polychaetes *Mediomastus ambiseta*, *Streblospio benedicti*, and *Polydora ligni* (Grassle et al. 1985; Widbom and Elmgren 1988). During 1982–83, a benthic predator, *Crangon septemspinosa*, severely reduced these populations in some treatments (J. Frithsen, Versar Incorp., Columbia, MD, unpubl. data) and only then did copepod numbers increase (Fig. 1b, top panel). An exponential increase in numbers of copepods during year 2 was correlated with decreasing abundance of benthic macrofauna ($r^2 = 0.806$).

These correlative data implied that other groups may outcompete the holozooplankton, especially in the presence of newly produced carbon. A third study (1985–86, sediment by nutrient interaction) was therefore designed to specifically probe the role of the benthos and its influence on pelagic community structure in which comparisons could be made across both control and enriched treatments with and without sediments. Results of this study have been published (Doering et al. 1989; Sullivan and Banzon 1990) but here we wish to review some pertinent points and present additional information. The most surprising and clear-cut result was that structure of the zoo-

TABLE 2. Average numbers of adult copepods, ctenophores, and benthos in control mesocosms (no nutrients added). Data are from a complete annual cycle. Average numbers at the source station in Narragansett Bay were computed over the same period and compared with mesocosm numbers. The standard deviation was calculated from replicate mesocosms. For 1979–80, sediments came from (1) Providence River, (2) mid-Bay, and (3) Rhode Island Sound. For 1985–86, Sed. = controls with sediment, No sed. = controls without sediment.

	Copepods ($no \cdot m^{-3} \pm SD$)	Macrofauna ($no \cdot m^{-2} \pm SD$)	Ctenophores ($no \cdot m^{-3} \pm SD$)
1979–80			
Mesocosms			
1	8027 ± 866	7 600	0.33 ± 0.17
2	5606 ± 1012	10 800	0.09 ± 0.02
3	11 518 ± 2587	11 600	0.02 ± 0.02
Bay	2364		0.03
1981–82			
Mesocosms	3435 ± 1342	62 788 ± 10 228	0
Bay	2179		0.08
1982–83			
Mesocosms	2019 ± 863	59 979 ± 15 889	0
Bay	1967		0.15
1985–86			
Mesocosms			
Sed.	5187 ± 1608	49 019 ± 2798	0.86 ± 0.86
No. sed.	2443 ± 255	Not present	35.0 ± 3.34
Bay	750		23.81

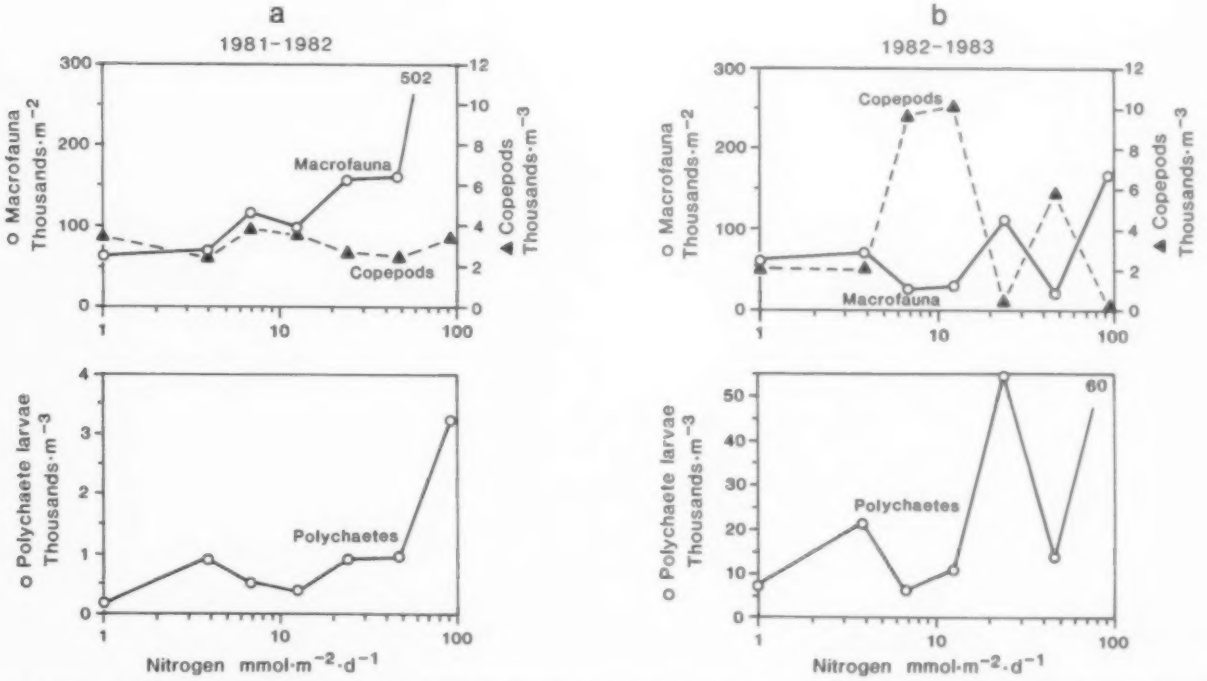

FIG. 1. Response of macrobenthos, copepods, and meroplankton to nutrient enrichment of mesocosms. Average numbers during 2 successive yr of a 2.5-yr-long experiment are shown. Standard deviation for the mean of control treatments is contained in Table 1.

plankton community was more dependent on the presence or absence of benthos than on nutrient input (Fig. 2; Table 3). Nutrient addition did increase the concentration of chlorophyll *a*, numbers of diatoms, and total zooplankton biomass (Table 3), while the sediment treatment had no significant effect on any of these parameters. However, sediment treatment had a very pronounced affect on community structure of the zooplankton (Table 3). Numbers of the carnivorous groups, chaetognaths (*Sagitta elegans*), ctenophores (*Mnemiopsis leidyi* and *Pleurobrachia sp.*), jellyfish (*Aurelia, Obelia, Rathkea*), and

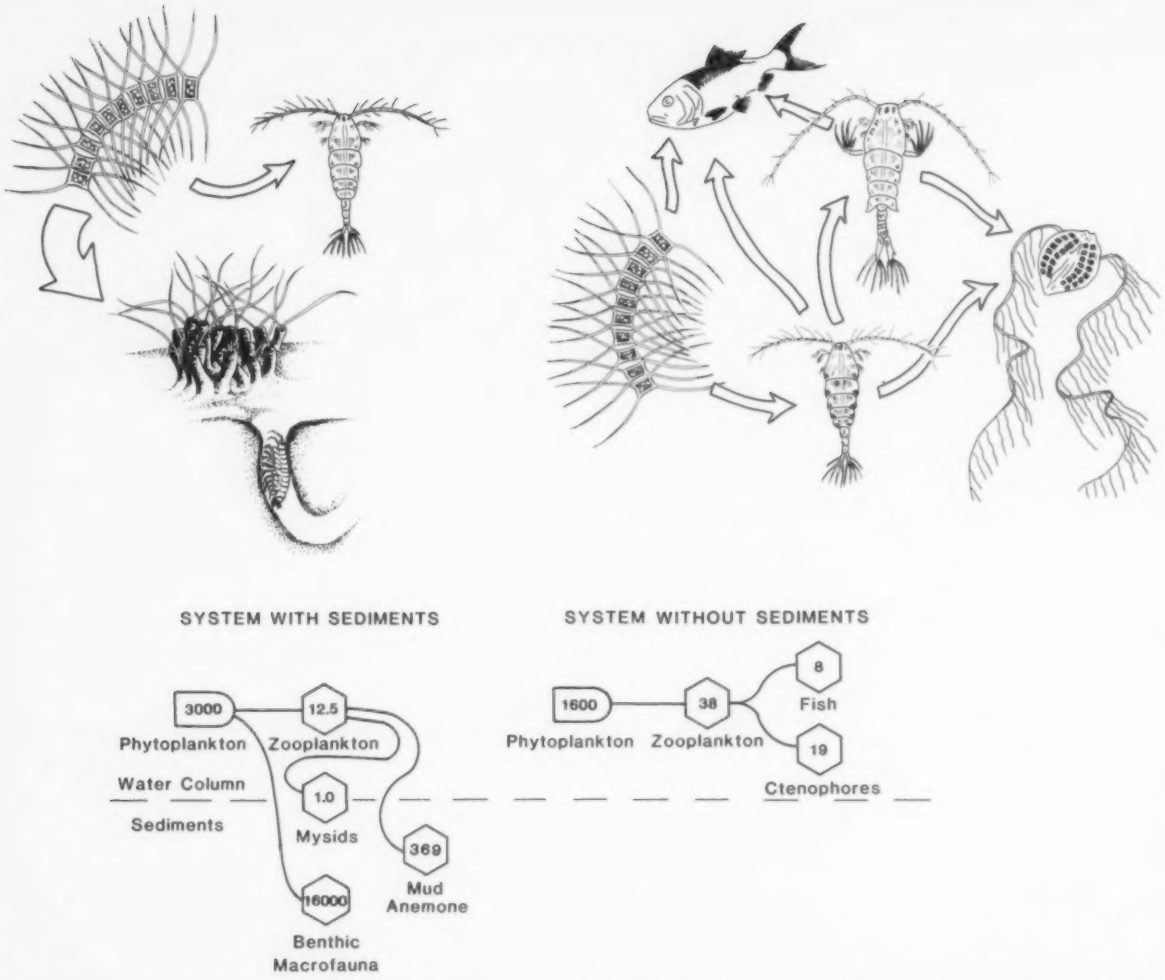

FIG. 2. Trophic interactions in enclosures with and without sediments. Top: idealized view of food web; bottom: average biomass of selected trophic groups in control tanks during the fish month of the 1985–86 experiment. Values are mg $C \cdot m^{-2}$. Dry weight biomass values have been converted to carbon according to published methods (Frithsen et al. 1986; Ovaitt et al. 1984). Additional data from this experiment are contained in Table 2.

a pelagic fish species (Atlantic menhaden) were significantly more abundant in all enclosures without sediment than in sedimented tanks.

It is clear that total animal biomass in the water column (zooplankton and fish) of all no-sediment treatments was higher than in sedimented enclosures (Table 3). The rapidity with which this difference developed was striking and is illustrated by the food web which developed only 1 mo after the experiment had begun (Fig. 2). However, fish were not present during the whole experimental period (Fig. 3) and it is likely that the community structure in the water column was heavily influenced by fish predation during the period when they were present. Before the larval fish were detected, copepod nauplii were very abundant in mesocosms without benthos (Fig. 4). Since nauplii are an important food item in the diet of early larval Atlantic menhaden (June and Carlson 1971), this initially large abundance may have promoted survival of the fish. However, after the first month, and following the first appearance of Atlantic menhaden larvae, numbers of copepods in the no-sediment mesocosms were reduced (presumably by predation) to values equal to (in nutrient treatments) or below (in controls) those in the sediment (Fig. 3). After disappearance of the fish from the mesocosm (none survived cold winter temperatures), numbers of copepods in the no-sediment treatment rebounded, and again surpassed numbers in mesocosms with sediment (Fig. 3).

Discussion

The benthos may have affected pelagic communities during these experiments through direct herbivory by filter-feeding benthos, predation of larvae by benthic adults, or by competitive, predatory, or otherwise inhibitory interactions between meroplankton and holoplankton. It is somewhat surprising that these interactions have received so little attention despite considerable knowledge of them from the field. For example, an

TABLE 3. Response (annual mean) of various trophic groups to sediment and nutrient manipulations of eight enclosures during June 1985 to June 1986. Values are the mean of two replicate enclosures for control and nutrient enriched treatments calculated by trapezoidal integration of time series data. Nutrient enriched treatments received 23.03 mmol N, 1.80 mmol P, and 1.64 mmol $Si \cdot m^{-2} \cdot d^{-1}$. Statistically, data were evaluated using a three-factor nested ANOVA. P_s = probability of a sediment effect; P_n = probability of a nutrient effect.

Trophic group	With sediment	No sediment	P_n	P_s
A. Control, no nutrient addition				
Chlorophyll *a* ($\mu g \cdot L^{-1}$)	6.6	4.8	0.0001	0.5313
Diatoms ($no \cdot mL^{-1}$)	860.0	713.9	0.0063	0.3450
Zooplankton (mg dry $wt \cdot m^{-3}$)	39.4	46.6	0.0203	0.7700
Copepods (10^3 individuals$\cdot m^{-3}$)	21.0	21.6		
Carnivorous holoplankton ($no \cdot m^{-3}$)	6.8	120.4	0.0028	0.0001
Fish (*Brevoortia tyrannus*)				
(mg dry $wt \cdot m^{-3}$)	0	384.6[a]		
Benthos (mg $C \cdot m^{-2}$)	2939[b]	—[c]		
B. 8× nutrient addition				
Chlorophyll *a*	28.5	28.6		
Diatoms	4473.5	3372.5		
Zooplankton	112.9	117.6		
Copepods	34.7	67.2		
Carnivorous holoplankton	9.1	59.4		
Fish	0	1017.0[a]		
Benthos	5948	—[c]		

[a]Abundance of fish was similar in both control and nutrient treatments. The higher biomass was due to large fish in the nutrient treatments.

[b]This value does not represent the amount produced during the experimental period because a large biomass existed at the start.

[c]Not measured, negligible biomass accumulated.

abundant literature attests to the fact that benthic filter feeders consume phytoplankton directly (Winter 1978), and in eutrophic waters, meroplankton have been observed to outnumber holoplankton and to utilize available food resources at a higher rate than holoplankton (Turner and Anderson 1983). Thus, competitive interactions are possible. Predation by adult benthic invertebrates on free-swimming pelagic larvae of the benthos is also a well-known phenomenon (Mileikovsky 1974). It is a small step to hypothesize that benthic predation is likely to affect holozooplankton and there are some observations of this interaction (Daro and Polk 1973; Dauer 1983; Eleftheriou and Basford 1983). However, the significance of these benthic groups as plankton predators is not well known and represents an area in which further study is needed.

Besides the mechanisms proposed above, several alternative explanations for benthic suppression of the holoplankton exist. First, the presence of the sediment itself rather than the benthic biota may have limited survival or productivity of water column species. We have published a detailed account comparing growth and reproductive rates of copepods in mesocosms with and without benthos which argues against this possibility (Sullivan and Banzon 1990). Second, the very presence of the fish themselves may have been the dominant force in producing the water column response in mesocosms without benthos, rather than the direct activity of the benthic fauna. We do not believe this to be the case based on our observations during periods with and without fish. Other groups of holozooplankton were also less abundant in mesocosms with benthos, some while fish were present (ctenophores) and others only when fish were absent (copepods and medusans). Nevertheless, it does appear that the fish had some affect on copepod abundance, based on different copepod responses during periods when fish were present versus when absent.

The suppression of higher trophic levels in the zooplankton and the limitation of the copepod response in mesocosms with a benthos are results consistent with hypotheses that the benthos can be an important force in molding estuarine structure and dynamics. While these interactions have been predicted from patterns observed in at least several field settings (e.g. San Francisco Bay and the Bay of Fundy; Cloern 1982 and Daborn 1986), the correspondence between mesocosm results and these predictions suggests a more general importance in a variety of estuaries, both nutrient limited and nutrient enriched. Therefore, it becomes important to test these experimental results in natural systems. This iterative process should be clarified by a comparison of physical, chemical, and biological characteristics and behavior of the mesocosms with natural systems.

To some extent, physical design of mesocosms must dictate chemical and biological responses. Surface to volume ratio is necessarily higher in enclosures than in nature. Light fields also vary somewhat from natural systems, but a major factor, light extinction with depth, has been matched to that in Narragansett Bay. Mixing is controlled by movement of a vertical plunger which produces a well-mixed system with a suspended sediment load in the water column similar to that in the Bay, albeit with a larger vertical component to the mixing than at the comparison station in the lower Bay (Nixon et al. 1980). However, despite these differences between the mesocosm and the Bay, chemical and biogeochemical cycles are remarkably similar (Pilson 1985b; Santschi et al. 1982). With regard to species composition and population biology, there are also many remarkable similarities. There has always been good agreement between seasonal dynamics of the natural communities and those in the mesocosms. Comparisons for the benthos have been reported by Grassle et al. (1981), Frithsen (1984), and Widbom and Elmgren (1988). Timing of appearance and disappearance

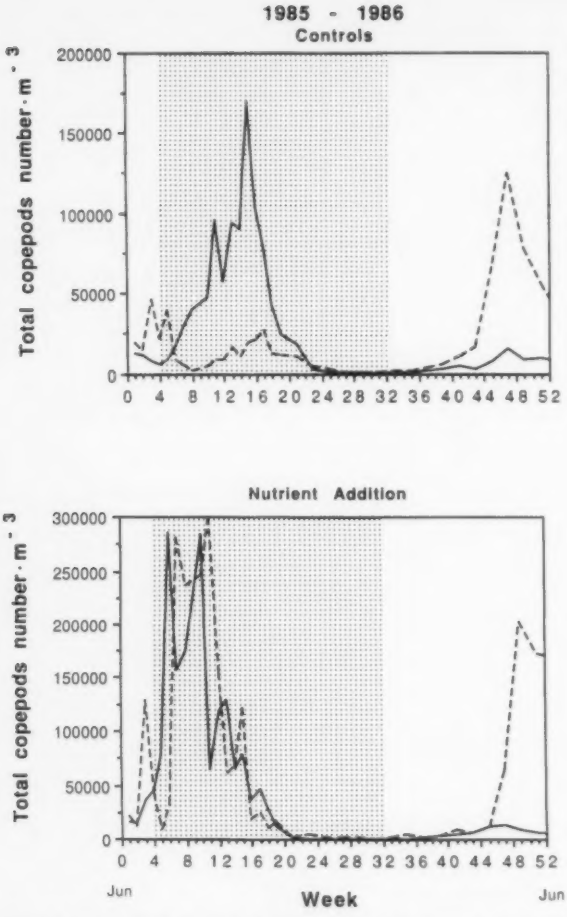

FIG. 3. Presence of fish and abundance of total copepods during the 1985–86 sediment (—)/no-sediment(---) experiment. Time of first and last appearance of larval *Brevoortia tyrannus* (in the no-sediment treatment only) is indicated by stippling. Mean of two replicate mesocosms for each treatment is plotted.

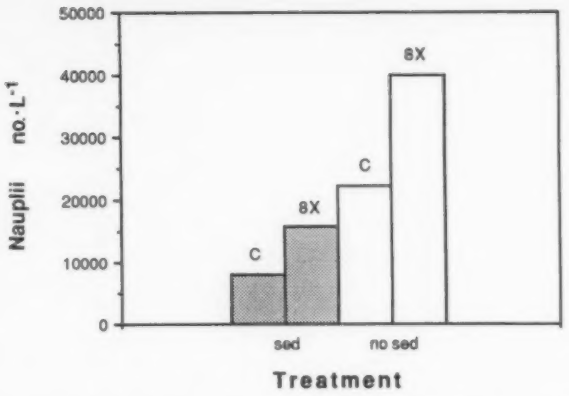

FIG. 4. Abundances of nauplii in enclosures during the first month of the 1985–86 experiment. Data are means calculated from time series data by a trapezoidal integration. Mesocosms with sediments are represented by stippled bars and those without sediments by open bars. Nutrient treatment (8×) is detailed in Table 2.

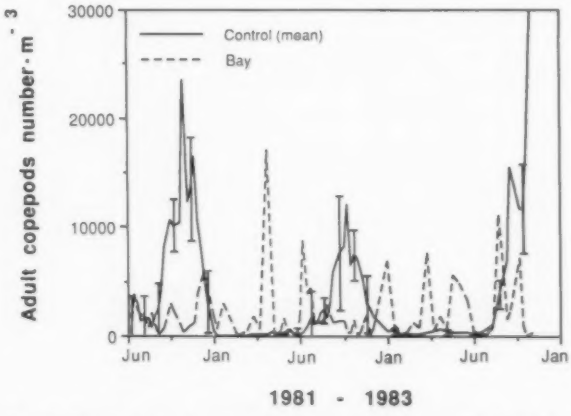

FIG. 5. Comparison of seasonal patterns of adult copepod abundances between mesocosms and Narragansett Bay during 1981–83. Mean and standard deviation of three replicate control mesocosms are plotted to demonstrate summer increase over Bay numbers.

of dominant copepod species is similar in the mesocosms and the lower Bay from which the mesocosms receive feed water (Sullivan and McManus 1986). An encouraging view of similarity between the Bay and mesocosms comes from a synoptic study comparing stable carbon isotope ratios of phytoplankton, zooplankton, and benthos in both systems (Gearing et al. 1984). The fact that no significant Bay–mesocosm differences were found implies that trophic dynamics in the mesocosms do not deviate appreciably from those in nature.

While it is encouraging to find Bay–mesocosm similarities, it is also important to recognize the differences and understand how they affect experimental results, as is always necessary in extrapolating from laboratory data to natural systems. One of the most persistent divergences between the mesocosms and the lower Bay comparison station became apparent after improving collection techniques for the benthos. While community composition and abundances of benthos then became similar to those at the source station, some consistent differences between Bay and mesocosm zooplankton became evident. Ctenophore numbers were often lower than expected from Bay samples, while copepod abundances became more similar to the Bay on an annual basis (Table 2). However, during the summer only, copepod numbers remained very much higher than in the Bay (Fig. 5). It is during this season that ctenophore predation is most pronounced, suggesting that these factors may be related: high numbers of macrofauna are correlated with low ctenophore numbers, and increases in the summer copepod population. Why are ctenophore numbers in mesocosms reduced below those in the lower Bay? We postulate one scenario with which the data are consistent. Assuming that mesocosm results are correct in showing that macrobenthos may reduce numbers of ctenophores and other gelatinous zooplankton (through predation, competition, or both mechanisms), then the rate of contact between organisms in the benthos and water column should control the rate of these interactions. The rate at which benthic filter feeders and predators can consume plankton must be related to processes such as mixing and stratification. As mentioned earlier, vertical mixing in the mesocosms is somewhat

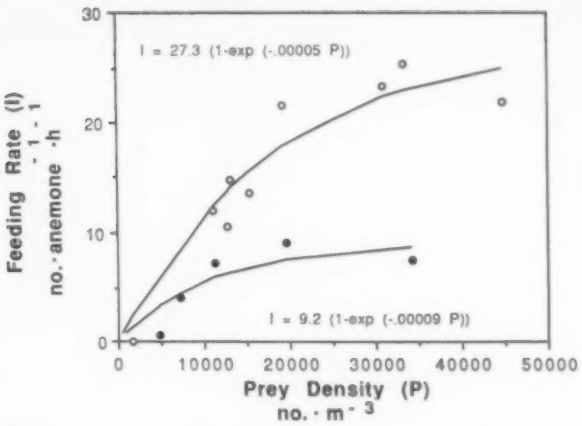

FIG. 6. Feeding rates of the mud anemone (*Ceriantheopsis americanus*) on zooplankton (copepod nauplii, copepodites, adults, and polychaete larvae). Open circles: rates from experimental containers (0.35-m^3 volume) mixed vertically with a plunger; closed circles: rates from containers without mixing. Data were fitted to an Ivlev curve using a nonlinear model in SAS and predicted values were used to create the plotted curve.

greater than in the Bay. If this factor in the design of the mesocosms tends to increase the rates of processes by which the macrobenthos inhibits ctenophore recruitment relative to these rates in nature, reduced abundances in the mesocosms would be explained. Results from earlier studies at the Marine Ecosystems Research Laboratory in which the effect of mixing versus stratification has been studied indicate that abundances of copepods and/or ctenophores increases when vertical mixing is reduced relative to the normal schedule (Oviatt 1981) or when horizontal mixing is used to create a stratified water column (Donaghay and Klos 1985). Although neither Oviatt (1981) nor Donaghay and Klos (1985) had conclusive explanations for the increase in zooplankton numbers, there is one interpretation that is consistent with the ideas in this paper: a reduction in vertical mixing (which should decrease mean flow across the bottom) reduces opportunities for contact between organisms in the water column and the benthos. In fact, the maximum feeding rate of at least one benthic predator commonly found in the mesocosms can be shown to be directly related to the amount of vertical stirring (Fig. 6).

In the field, factors that would control the mean flow across the benthos include water column depth, tidal mixing, and degree of water column stratification produced by freshwater input to an estuary. In Narragansett Bay, the water column is always stratified near the Providence River where freshwater supply produces a halocline. This effect becomes progressively reduced toward the Bay mouth, and in some shallow embayments the water column is almost always well mixed. The point is that the strength of hydrodynamic coupling in natural systems varies spatially, and in some systems temporally. We suggest that variability in water column communities is also, in part, a function of this effect. For instance, ctenophore abundance varies widely in the Bay but there is a consistent pattern in that their population develops first in the upper Bay and then is carried to the lower Bay (Kremer and Nixon 1976). Martin (1965) observed higher numbers of medusans and also of the copepods *Acartia tonsa* and *Acartia hudsonica* (called *clausi* by Martin) in the upper Bay. Durbin and Durbin (1981) also found this same pattern for adult *Acartia*.

In Narragansett Bay, there is also a gradient in nutrient concentration that correlates with changing hydrodynamics from the head to the mouth of the estuary. Thus, it is not possible to distinguish between the effects of these two factors on plankton abundance in different areas of the Bay. In mesocosms, the effects of nutrients and mixing can be examined separately. Our observations are consistent with the idea that, in areas where the water column is stratified, the benthos may have less influence over the water column community of holoplankton. They also suggest that, while the mesocosms are not scaled (in terms of amount of vertical mixing) to match the lower Bay comparison station exactly (in that the intensity of mixing may accentuate benthic–pelagic association), they are useful tools in identifying natural processes which control food web dynamics in estuaries.

The results are indicative of important interactions between benthos and water column that warrant consideration in the study of estuarine dynamics. In shallow, unstratified waters, the primary trophic link may be benthic rather than pelagic and this may dictate impoverished pelagic food webs. Thus, the strength of benthic–pelagic coupling in natural systems will determine which subsystem responds to eutrophication. These conclusions need to be tested at appropriate field sites where either geographic or temporal changes in water column structure allow observation of different degrees of benthic – water column coupling. In the mesocosms, experiments should involve experimentation with stratified and mixed systems with and without sediments. The effects of different types of mixing and the breakdown of water column stratification also need further analysis.

Acknowledgements

This work was supported by the U.S. Environmental Protection Agency's Centers Program CR 812487-03-0 and the Andrew Mellon Foundation. We thank Dr. T. A. Napora for drawing Fig. 2.

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Epiphyton Biomass is Related to Lake Trophic Status, Depth, and Macrophyte Architecture[1]

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The relationship between epiphyton biomass and water column total phosphorus concentration (TP) was studied in macrophyte beds in 11 lakes covering a wide range of trophic status (TP = 5.8–72.8 $\mu g \cdot L^{-1}$). Phosphorus concentration was a poor predictor of epiphyton biomass when considered alone. Our data do not agree with previous studies that found that epiphyton biomass increased continuously with TP. Instead, we found a very weak, nonlinear relationship between TP and epiphyton biomass, where epiphyton biomass increased up to TP $\approx$ 39 $\mu g \cdot L^{-1}$, and decreased at higher TP. Season and sampling depth accounted for significantly more variation in epiphyton biomass than did TP. Epiphyton biomass increased with depth in oligotrophic lakes but decreased with depth in eutrophic lakes. Seven common species of macrophytes of differing architecture developed significantly different epiphyton biomass. Macrophytes with flexible, ribbon-like leaves supported lower epiphyton biomass than species of broad-leaved or whorled architecture. The effect of host type on epiphyton algae biomass was not, however, as great as the influence of environmental variables.

Nous avons étudié la relation entre la biomasse d'épiphyton et la concentration de phosphore total (TP) dans des herbiers de macrophytes situés dans 11 lacs couvrant une vaste étendue d'états trophiques (TP = 5,8–72,8 $\mu g \cdot L^{-1}$). La concentration de phosphore, lorsqu'incluse seule dans l'analyse, s'est avérée un descripteur inefficace de la biomasse d'épiphyton. Nos données ne corroborent pas la relation positive et linéaire retrouvée entre TP et la biomasse d'épiphyton par d'autres auteurs. Nous avons trouvé une relation très faible et non linéaire, suggérant que l'effet du TP est positif jusqu'à une concentration de $\approx$39 $\mu g \cdot L^{-1}$, au-delà de laquelle la biomasse d'épiphyton diminue avec une augmentation de TP. Les variations saisonnières et la profondeur d'échantillonnage expliquent significativement plus de la variation en biomasse d'épiphyton que le TP. Ainsi, la biomasse d'épiphyton augmente avec la profondeur aux faibles concentrations de phosphore, mais elle diminue en conditions eutrophes. Nous avons observé des différences significatives de biomasse d'épiphyton sur les sept espèces de macrophytes de morphologie variée. Les macrophytes pourvues de feuilles en forme de rubans souples supportent de plus faibles biomasses d'épiphyton que les espèces à feuilles larges ou disposées en verticille. Cependant, l'effet du type de plante sur la biomasse d'épiphyton était moins grand que l'effet des variables environnementales.

Received September 14, 1990
Accepted May 28, 1991
(JA723)

Reçu le 14 septembre 1990
Accepté le 28 mai 1991

Epiphytic algae contribute significantly to the primary production of littoral zones colonized by aquatic macrophytes. For example, Kajak et al. (1972) found that epiphytic algae were responsible for 28% of the production in the littoral zone of Mikołajskie Lake, while Cattaneo and Kalff (1980) estimated that epiphytic algae contributed as much as 82% of the primary production in the littoral zone of Lake Memphremagog. Epiphytic algae are an important source of food for phytophilous invertebrates (Soszka 1975; Dvořak and Best 1982; Cattaneo 1983), which are a major food source for littoral fish and waterfowl (Keast 1984; Danell and Sjöberg 1980). The biomass of algae attached to substrates (periphyton, *f.* Wetzel 1983) has been suggested as a potential indicator of point-sources of nutrient enrichment (e.g. Patrick 1973; Ennis 1975; Collins and Weber 1978; American Public Health Association et al. 1985; Biggs 1989) because it is sensitive to variations in phosphorus availability and because it grows fixed to a physical substrate. Periphytic algae can become a nuisance, decreasing recreational use of water bodies (Horner et al. 1983). Although it is of practical and theoretical interest to determine factors limiting epiphyton biomass in lentic ecosystems, epiphytic algae are difficult to study because they have a heterogeneous spatial distribution (Pieczyńska and Szczepańska 1966; Brown and Austin 1973) and are difficult to separate from their macrophyte substrate (Cattaneo and Kalff 1978; Wetzel 1983).

Conflicting evidence exists about the relationship between periphytic algae and phosphorus. While single-lake studies and mesocosm enrichment experiments have found that periphyton biomass is influenced by nutrient concentration (Phillips et al. 1978; Cattaneo and Kalff 1980; Sand-Jensen and Søndergaard 1981; Krewer and Holm 1982; Fairchild et al. 1985; Osenberg 1989), interecosystem comparisons find weak correlations between phosphorus concentration and periphyton (Stevenson et al. 1985; Cattaneo 1987; Hansson 1988). Cattaneo and Kalff (1980) found that epiphyton biomass increased linearly with water column total phosphorus concentration (TP) in a single lake with a trophic gradient (TP from 10 to 40 $\mu g \cdot L^{-1}$). They further suggested (Cattaneo and Kalff 1980, p. 287) that the relationship they found could be used to predict the effects of phosphorus enrichment on the dynamics of macrophyte beds. Other authors (e.g. Moss 1976; Hansson 1988) have suggested, however, that phosphorus enrichment leads to decreased peri-

[1]Publication No. 373 of the Groupe d'Ecologie des eaux douces de l'Université de Montréal.

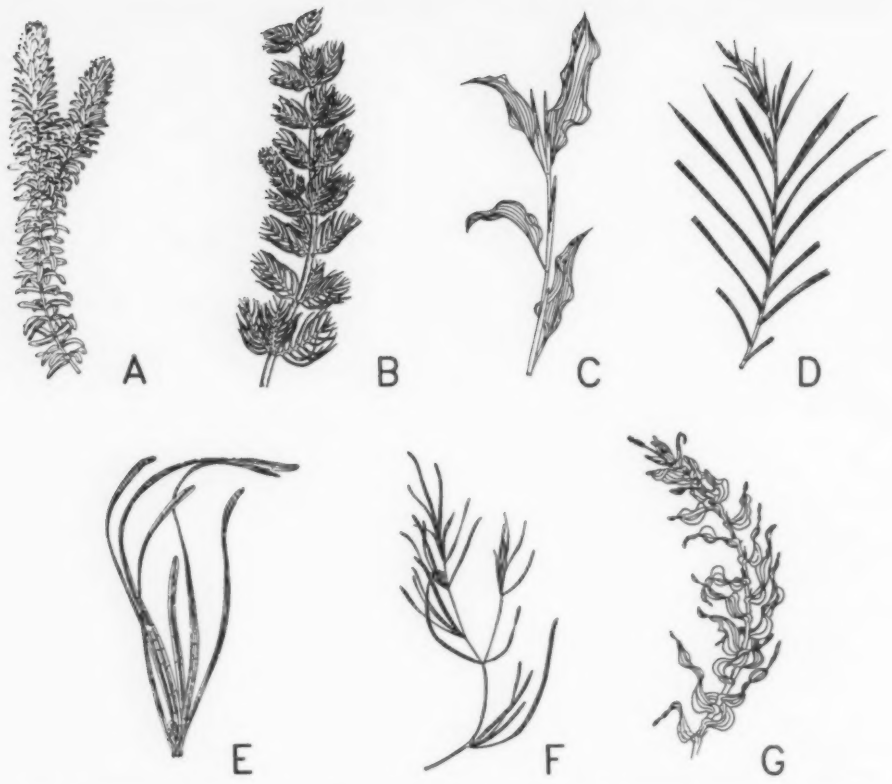

FIG. 1. Seven species of macrophytes sampled (from Fassett 1957). A, *Elodea canadensis*; B, *Myriophyllum spicatum*; C, *Potamogeton amplifolius*; D, *Potamogeton robbinsii*; E, *Vallisneria americana*; F, *Potamogeton* sp.; G, *Potamogeton richardsonii*.

phyton biomass because of light attenuation caused by dense phytoplankton growth at high TP. There is, therefore, reason to believe that correlations between epiphyton biomass and TP should not be linear, but that epiphyton biomass is reduced at high TP.

Factors other than nutrient concentration may also influence the biomass of algae growing on the surfaces of aquatic macrophytes. In a mesotrophic lake, on the one hand, Cattaneo and Kalff (1980) found that macrophyte beds in deeper waters supported higher epiphyton biomass, but that within one bed, the leaves of plants closest to the water surface had the greatest epiphyton productivity. On the other hand, Allen and Ocevski (1981) found that the vertical distribution of epiphyton abundance in an oligotrophic lake was uniform, while Young (1945) showed that epiphyton on deeper macrophytes in an oligotrophic lake was less subject to surface turbulence, and therefore more abundant than that found on shallower macrophytes. These results, taken together, suggest that epiphyton abundance at different depths will be influenced differently by factors such as light and turbulence and that these effects might be different, even reversed, in lakes of various trophic status.

Architecture of macrophytes might also influence epiphyton abundance. It is widely accepted that different macrophyte species support different amounts of epiphyton (Young 1945; Prowse 1959; Cattaneo and Kalff 1980; Allen and Ocevski 1981), and several authors have suggested that macrophytes with finely dissected leaves (e.g. *Myriophyllum* spp.) develop the greatest epiphyton biomass (Foerster and Schlichting 1965; Kowalczewski 1975; Cattaneo and Kalff 1980; Dvořak and Best 1982; Gregg and Rose 1982) because of their high surface to biomass ratio. We therefore expect that macrophyte species with finely dissected leaves should support the greatest epiphyton biomass. Few quantitative, interspecies comparisons have been attempted on submerged plants, however.

The first objective of this study was to test the hypothesis that TP and epiphyton biomass covary in a series of lakes of different trophic status. Such interlake studies of the periphyton–phosphorus relationship are rare (e.g. Cattaneo 1987, for epilithon; Stevenson et al. 1985 and Hansson 1988, for epipelon) and we know of no previous study of epiphyton that has tested this hypothesis. Next, we tested the hypothesis that macrophyte beds in deeper waters support higher epiphyton biomass. We also examined the simultaneous roles of light attenuation and turbulence by examining how epiphyte abundance covaries with the distance below the water surface in lakes of different trophic status. We hypothesize that epiphyton biomass should increase with depth below the water surface in oligotrophic lakes (due to the effect of turbulence) but should decrease with depth in eutrophic lakes (due to light attenuation by phytoplankton). Therefore, both total depth and depth below water surface should be positively correlated with epiphyton biomass, while the interaction between TP and distance from water surface should be negatively correlated with epiphyton biomass. Finally, we tested the hypothesis that the biomass of

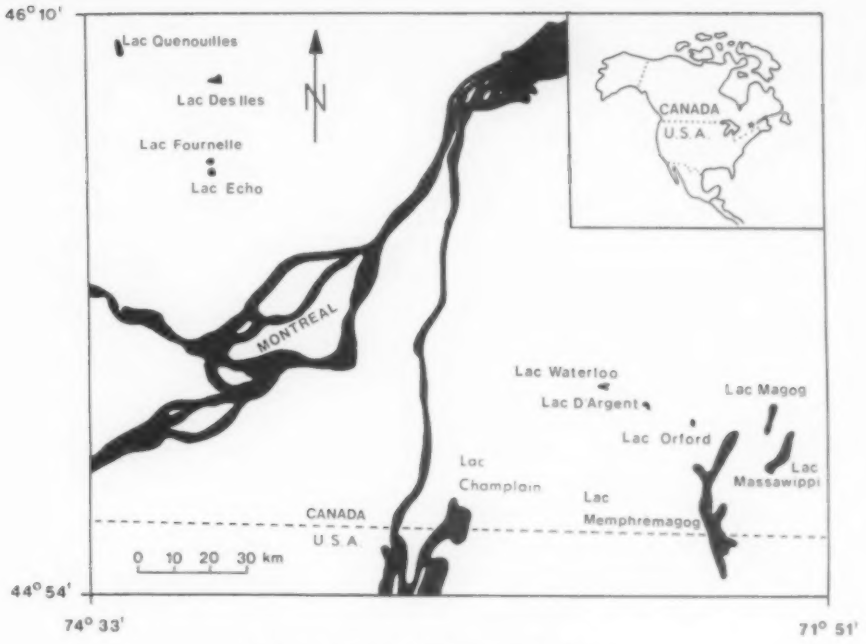

FIG. 2. Location of the 11 sampling sites.

epiphytic algae colonizing the surfaces of macrophytes varies significantly among macrophytes of differing architecture.

Methods

The epiphyton found on seven species of submerged aquatic macrophytes (Fig. 1) was sampled in 11 lakes in southern Québec, Canada (Fig. 2). Samples were collected throughout the months of July and August in two successive years. Lakes and macrophyte beds were selected to represent a wide range of macrophyte species diversity, epiphyton abundance, and trophic status in order to maximize our ability to detect significant effects of phosphorus concentration, water depth, and macrophyte architecture.

Samples of epiphyton were taken at random points along a 50-m transect installed parallel to the shore in one macrophyte bed in each lake. Between 20 and 30 samples of macrophytes were collected at each site using a 6-L Plexiglas box that was closed gently around macrophytes without disturbing the epiphytic flora and fauna (Downing 1986). At each site, samples were taken at different, randomly chosen distances below the water surface (Z_s, metres), i.e. at different distances from the top of the macrophytes which did not all grow to the water's surface. The total water depth (Z_t, metres) at each epiphyton sampling point was also noted. All samples were carefully collected by divers. After each sample was sealed in the plastic box sampler and brought to the boat, a random subsample of macrophyte and its epiphytes was withdrawn through an opening in the side of the sampler. Each epiphyton sample was then placed gently into a clean jar containing 200 mL of filtered lake water. All samples were refrigerated until laboratory analysis which was performed within 24 h of collection.

The epiphyton biomass (EPI, micrograms chlorophyll *a* per gram dry mass of plant) was determined using Cattaneo and Kalff's (1978) method. Each sample jar was vigorously shaken 100 times to dislodge the epiphytes from the macrophytes. This technique samples the loosely attached algal community without contaminating the samples with macrophyte tissue (Cattaneo and Kalff 1978). A subsample of 20–200 mL of epiphyte suspension was filtered (Millipore AP40 04705) and the filters were frozen. Chlorophyll *a* was later extracted from these filters by soaking them in 96% ethanol for 24 h in the dark and under refrigeration. This chlorophyll solution was subsequently filtered to reduce turbidity and was read spectrophotometrically (Spectronic 21, Bausch and Lomb) at wavelengths of 665 and 649 nm (Bergman and Peters 1980). Macrophytes from which epiphyton was removed were identified (Fassett 1957) and dried to constant mass (60°C; ± 0.1 mg).

A 1-L water sample was taken by a diver using an opaque, acid-washed plastic bottle approximately 30 cm beneath the water surface at 10 m intervals along each 50-m transect (six 1-L samples per site). TP (micrograms per litre) in this sample was determined by persulfate digestion followed by the ascorbic acid colorimetric method (American Public Health Association et al. 1985). Because variations in TP within macrophyte beds are generally very low (Table 1; Cyr and Downing 1988), TP estimates were not paired with epiphyton samples, but represented a macrophyte bed mean.

The literature suggests that, in general, epiphyton abundance is greatest in spring, stable or decreasing slowly throughout the summer, and increasing in autumn (Pieczyńska and Szczepańska 1966; Cattaneo and Kalff 1980; Cattaneo 1983). Our study was performed during the shortest possible period (July and August) to avoid as much seasonal variation as possible. The sampling date, however, was employed as an independent variable in the statistical analyses to ensure that seasonal variation would not confound analyses. These indices of season varied from 1 (first day of sampling) to 44 (last day of sampling).

TABLE 1. Average characteristics of 11 macrophyte beds sampled (standard deviation in parentheses). TP, total phosphorus concentration of the water surrounding the macrophytes; *S*, phytoplankton abundance (chlorophyll *a*) in each macrophyte bed; EPI, epiphyton biomass per unit dry mass of macrophyte; $\bar{Z}$, mean depth of sampling site. The principal macrophyte species that represented more than 1% of the total macrophyte standing biomass are listed in decreasing order of abundance for each macrophyte bed. Cd, *Ceratophyllum demersum*; Ch, *Chara* sp.; Cla, *Cladophora* sp.; Ec, *Elodea canadensis*; Hd, *Heteranthera dubia*; J, *Juncus* sp.; M, *Myriophyllum spicatum* (except in Lac Des Iles, where *M. humile* was found); N, *Nitella* sp.; Pa, *Potamogeton amplifolius*; Pr, *Potamogeton robbinsii*; Pric, *Potamogeton richardsonii*; Ppr, *Potamogeton praelongus*; P, *Potamogeton* sp.; S, *Sagittaria* sp.; Va, *Vallisneria americana*.

Lake	TP ($\mu g \cdot L^{-1}$)	*S* (μg Chl $a \cdot L^{-1}$)	EPI (μg Chl $a \cdot g^{-1}$)	$\bar{Z}$ (m)	Dominant macrophytes
Des Iles	5.8 (0.7)	1.5 (1.1)	480.3 (615.9)	1.6 (0.1)	M, Pa, Pr, Ni, P
Orford	6.9 (1.8)	1.5 (0.6)	235.4 (195.8)	2.0 (0.3)	Pa, Ec, Ni
Fournelle	7.0 (1.1)	2.6 (1.0)	466.1 (382.4)	1.6 (0.3)	Pr, Ec
Quenouilles	11.2 (2.8)	1.6 (0.6)	217.3 (218.6)	1.3 (0.1)	Pa, J, Ch, S, Va
Echo	12.8 (5.8)	8.3 (7.6)	141.5 (127.1)	1.6 (0.1)	Ch, Pa, Pr, Ppr
D'Argent	13.2 (2.6)	7.5 (2.3)	209.1 (164.4)	1.5 (0.3)	M, Ni, Va
Memphremagog (Cove Island Bay)	17.5 (3.9)	3.3 (0.8)	114.0 (82.5)	2.2 (0.1)	M, Va, Hd, Ec
Champlain (Baie de Venise)	23.3 (4.1)	3.5 (2.3)	120.0 (88.7)	2.0 (0.0)	Va, Cla, Pric
Massawippi	23.9 (9.2)	15.9 (12.4)	995.4 (484.2)	2.2 (0.4)	M
Magog	57.5 (20.5)	19.8 (12.9)	413.7 (310.4)	1.6 (0.1)	Ec, M, Pr, Cd
Waterloo	72.8 (17.4)	41.7 (11.0)	121.6 (52.0)	1.1 (0.1)	M, Va

The correlation between epiphyton biomass and phosphorus concentration was tested by calculating Pearson's correlation coefficient (r) between epiphyton biomass and TP. We tested the hypothesis that epiphyton biomass and TP were nonlinearly related with polynomial regression analysis. Bivariate correlations between epiphyton biomass and macrophyte bed characteristics were examined using Pearson's correlation coefficient. The hypothesis that several of these characteristics might influence epiphyton biomass simultaneously was further tested by multiple, linear, stepwise regression analysis. Candidate variables in this analysis were total phosphorus concentration (TP), water depth (Z_t), distance between sample and water surface (Z_s), and the sampling date (D). In addition to these variables, we included the square of the total phosphorus concentration (TP^2) to test for the probable negative influence of high concentrations of phosphorus on epiphyton biomass. We also included the interaction term of phosphorus and distance below water surface ($TP \cdot Z_s$) to test for the reversal of the effect of depth on epiphyton biomass at high TP. Because measures of TP were not directly paired with epiphyton estimates, we followed Draper and Smith's protocol (1981, p. 241) for the calculation of regression coefficients, degrees of freedom, and significance of effects when using dummy variables with more than two levels (i.e. TP). An analysis of variance and a multiple comparisons test (Student–Newman–Keuls) were subsequently performed on the residuals of the multiple regression to determine whether the epiphyton biomass differed among species of aquatic macrophytes after accounting for the effects of season, lake trophic status, and depth. Epiphyton data were transformed to their fourth root for entry into polynomial and multiple regression analyses to normalize residuals. All statistical analyses were performed using SPSS.

Results and Discussion

The TP of the 11 study lakes varied between 5.8 and 72.8 $\mu g \cdot L^{-1}$ (Table 1), ranging from oligotrophic to eutrophic conditions (Wetzel 1983). Variation of TP within macrophyte beds was low (Table 1). Although Cattaneo and Kalff (1980) found a linear relationship between epiphyton biomass and TP, our results (Fig. 3) indicated that, in our range of TP, there was no significant relationship between these variables ($r = -0.066$, $p = 0.85$, $n = 11$), and in the range of TP observed by Cattaneo and Kalff (1980) ($<40 \mu g \cdot L^{-1}$), the weak positive correlation we observed between TP and epiphyton biomass was not statistically significant ($r = 0.21$, $p = 0.58$, $n = 11$). At high TP, epiphyton biomass appeared to decrease, but a polynomial regression analysis performed on macrophyte bed

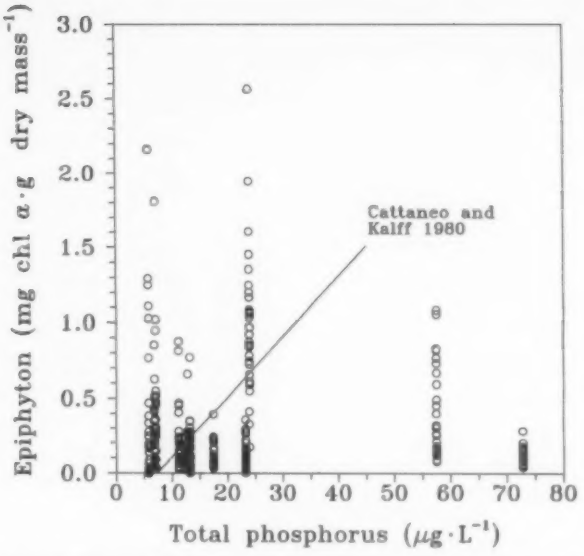

FIG. 3. Relationship between epiphyton biomass and TP in the 11 lakes sampled. The line represents the relationship found by Cattaneo and Kalff (1980) ($y = 0.44x - 0.29$; $r = 0.85$).

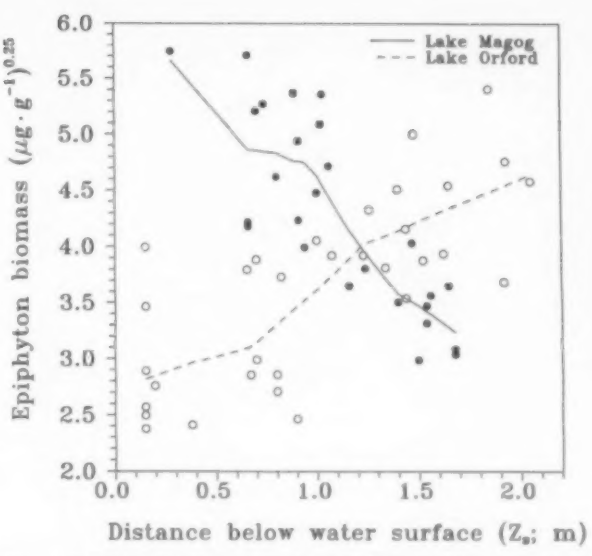

FIG. 4. Relationship between transformed epiphyton biomass and the depth below water surface at which samples were taken for two different values of TP: Lake Orford (7 $\mu g \cdot L^{-1}$) and Lake Magog (58 $\mu g \cdot L^{-1}$). The Lowess fit (robust locally weighted regression; Cleveland 1979) is shown for each lake.

TABLE 2. Pearson correlation coefficients (r) for bivariate relationships between epiphyton biomass and characteristics of sampling sites. Correlations using D and TP were performed using means.

Variable	r	n	p
Sampling date (D)	−0.46	11	0.158
Total phosphorus (TP)	−0.07	11	0.848
Distance of sample from water surface (Z_s)	0.27	260	0.001
Total water depth (Z_t)	0.26	280	0.001

TABLE 3. Mean biomass (standard deviation in parentheses) of epiphyton biomass measured on seven species of aquatic macrophytes. The averages were calculated from all available data for each macrophyte species over all 11 lakes in which it was found.

Macrophyte species	Number of lakes	Number of observations	Epiphyton (μg Chl $a \cdot g^{-1}$)
Elodea canadensis	3	13	494.2 (520.3)
Myriophyllum spicatum	7	121	487.1 (513.7)
Potamogeton robbinsii	3	15	266.6 (140.6)
Potamogeton amplifolius	5	90	200.5 (175.5)
Vallisneria americana	3	25	146.8 (90.6)
Potamogeton sp.	1	11	88.8 (97.8)
Potamogeton richardsonii	1	5	63.3 (68.3)

average values of epiphyton biomass and TP ($n = 11$) indicated that this relationship was not statistically significant ($p = 0.85$). Our results therefore disagree with Cattaneo and Kalff's (1980) hypothesis that eutrophication results in a linear increase in epiphyton biomass. Although this is an unexpected result for epiphytic algae, other interlake comparisons of epipelic (Stevenson et al. 1985; Hansson 1988) and epilithic (Cattaneo 1987) algae have found weak, often conflicting relationships between periphyton biomass and TP.

The lack of relationship between epiphyton biomass and TP was not due to a lack of phosphorus limitation in the lakes that we studied, as the logarithm of the concentration of chlorophyll *a* in the suspended phytoplankton was significantly correlated with log TP (Lalonde 1988: $r^2 = 0.75$, slope = 1.2) in a way that was similar to that found elsewhere in the littoral zone (Cattaneo 1987: $r^2 = 0.89$, slope = 1.33) and in the pelagic zone (Pace 1984: $r^2 = 0.93$, slope = 1.09; Dillon and Rigler 1974: $r^2 = 0.90$, slope = 1.45).

Epiphyton biomass varied widely at all phosphorus concentrations (Fig. 3), suggesting that variables other than phosphorus had a significant impact on epiphyton biomass (Table 2). Multiple regression analysis showed that epiphyton biomass (EPI) varied as

$$(1)\quad (\mathrm{EPI})^{0.25} = -0.0435(\mathrm{D}) + 1.1412(Z_s) - 0.0013(\mathrm{TP}^2) + 0.1151(\mathrm{TP}) - 0.0367(\mathrm{TP}\cdot Z_s) + 3.1002$$

($R^2 = 0.40$, $F = 34.2$, $p < 0.0001$, $n = 260$) where D is the sampling date, Z_s is the distance between the water surface and the sample, TP is the concentration of total phosphorus in the water column, and $\mathrm{TP}\cdot Z_s$ is the product of TP and the depth below the water surface at which the sample was taken. All of the regression coefficients in Eq. 1 differ significantly from zero ($p < 0.0001$) and the variables are listed in decreasing order of the significance of their partial t-values. The total water depth (Z_t) at each epiphyton sampling point had no significant effect.

Equation 1 shows that after accounting for the significant effects of other variables by multiple regression, TP had a weak

but significant positive effect on epiphyton biomass up to 39 $\mu g \cdot L^{-1}$, above which epiphyton biomass decreased (negative effect of TP^2). Equation 1 reconciles the conflicting results of Cattaneo and Kalff (1980) and Hansson (1988). Within the range of low phosphorus concentrations studied by Cattaneo and Kalff (10–40 $\mu g \cdot L^{-1}$), our results agree with the positive correlation between epiphyton biomass and TP that they observed. Our analysis of Hansson's (1988) data showed that epipelon biomass decreased at high TP ($p = 0.052$) in his 20 study lakes only when two lakes with TP > 100 $\mu g \cdot L^{-1}$ were considered.

The sign of the coefficient for the TP·Z_s interaction term shows that the form of the relationship between epiphyton biomass and sampling depth depends on TP. Therefore, at low TP, epiphyton biomass increases with distance below the water surface whereas at high TP, this relationship is reversed due to light attenuation, and higher biomasses of epiphyton are found closer to the water surface (Fig. 4). The lower biomass of epiphyton closer to the water surface in oligotrophic lakes could be attributable to shorter colonization times on younger parts of macrophytes, or greater turbulence at the surface. Evans and Stockner (1972) have observed similar reversed relationships between depth and epiphyton biomass in oligotrophic versus eutrophic lakes.

Season explained the most variance in epiphyton biomass, even considering data from lakes of different trophic status, sampled within a short period of time. As in other studies (e.g. Pieczyńska and Szczepańska 1966; Cattaneo and Kalff 1978, 1980; Sand-Jensen 1983), epiphyton biomass decreased as the season progressed. This systematic decrease of epiphyton during the summer has been linked to grazing by phytophilous invertebrates (Cattaneo 1983; Kairesalo 1984). Besides grazing, other factors such as nutrient depletion (nitrogen and silicon), temperature and light variations, and macrophyte age and condition could be linked to seasonal variations in epiphyton biomass.

The seven macrophyte species that we collected (Fig. 1) did not support an equal biomass of epiphyton when expressed per unit dry mass of macrophyte (Table 3). Our results agree with those of other authors who found that different macrophyte species were colonized differentially by epiphytic algae (Kowalczewski 1975; Cattaneo and Kalff 1980; Allen and Ocevski 1981; Gregg and Rose 1982). None of the seven macrophyte species that we studied was present in all lakes (Table 3); thus, apparent differences in epiphyton biomass on different macrophyte species could have arisen from different environmental characteristics prevailing at the different sites colonized by these macrophyte species.

Analysis of the residuals of Eq. 1 shows that the different macrophyte species supported significantly different biomasses of epiphyton, even after compensation for the effects of lake trophic status, depth, and season (ANOVA: $F = 6.486$, $p < 0.0001$; Table 4). Average residuals appear to be related to macrophyte architecture: the two whorled macrophytes *Myriophyllum spicatum* and *Elodea canadensis* had the highest residuals, the broad-leaved macrophyte *Potamogeton amplifolius* had the next highest residuals, and the ribbon-leaved macrophytes had three of the four lowest residuals (Table 4). The only outlier to this pattern was *Potamogeton richardsonii* which was sampled in only one lake ($n = 5$) and had very young leaves (i..e green leaves with no signs of senescence), which are often associated with low epiphyton biomass (Sand-Jensen and Søndergaard 1981; Rogers and Breen 1983; Sand-Jensen 1983). A Student–Newman–Keuls multiple comparisons test (Table 4) showed that only *Vallisneria americana*, *Potamogeton* sp., and *P. richardsonii* had epiphyton biomasses that were significantly different from those found on other species. None of the other architecturally diverse macrophyte species supported significantly eccentric epiphyton biomass. Therefore, even though plants such as *Myriophyllum* and *Elodea* seemed to have more abundant epiphyton than other macrophyte species (Table 3), part of the difference in colonization of the different macrophyte species appeared to reflect specific characteristics of different habitats in which certain macrophyte species grow (Tables 1 and 3). Once these specific characteristics were accounted for, differences in epiphyton biomass were less obvious. Thus, environmental and seasonal factors seemed to have a greater influence on epiphyton biomass than the architecture of the macrophyte species on which it grows.

This study concludes, therefore, that several factors, including lake trophic status, influence the development of epiphyton biomass. The macrophyte bed characteristics that we measured explained 40% of the variation in transformed epiphyton biomass, and a small additional amount of the residual variance was explained by differences in epiphyton biomass among seven macrophyte species. Our multivariate analyses showed that phosphorus concentration accounted for only a very small fraction of the total variation in epiphyton biomass in 11 lakes (Eq. 1) and that this relationship was nonlinear, showing a decrease in epiphyton biomass at high phosphorus concentrations. The effect of phosphorus enrichment was, however, to decrease light penetration in the water column, making it

TABLE 4. Tests for differences in the epiphyton biomass found on several species of macrophytes, made using a Student–Newman–Keuls multiple comparisons test on the residuals of Eq. 1. *Significant difference ($p < 0.05$); ns, no significant difference. Where significant differences exist, the greater epiphyton biomass was found on the species with the greatest mean residual. Sample size (n) is not the same as in Table 3 because some values of Z_s are missing (see Eq. 1).

	Myriophyllum spicatum	*Elodea canadensis*	*Potamogeton amplifolius*	*Vallisneria americana*	*Potamogeton robbinsii*	*Potamogeton* sp.	*Potamogeton richardsonii*
Myriophyllum spicatum		ns	ns	*	ns	*	*
Elodea canadensis			ns	ns	ns	*	ns
Potamogeton amplifolius				ns	ns	*	ns
Vallisneria americana					ns	*	ns
Potamogeton robbinsii						ns	ns
Potamogeton sp.							ns
Mean residual	0.2390	0.0919	−0.0561	−0.2012	−0.3925	−0.9920	−1.0230
Standard deviation	0.7923	0.9307	0.8778	0.6157	0.6309	0.5322	0.7990
n	117	11	82	23	12	10	5

impossible for epiphyton to grow at depth. Therefore, the pattern of increasing epiphyton biomass with distance below water surface in oligotrophic lakes was reversed in more eutrophic lakes. Although this model has a coefficient of determination too low to be useful in making predictions, it demonstrates that epiphyton biomass is more appropriately described by multivariate analysis and, unlike phytoplankton, cannot be simply predicted from TP, a finding corroborated by studies of other periphytic algae.

Acknowledgements

We thank H. Harvey, M. Pérusse, and G. Dagenais for field assistance, C. Plante for statistical advice, and A. Cattaneo, L.-A. Hansson, R. R. Horner, C. W. Osenberg, and an anonymous reviewer for their suggestions on the manuscript. This research was supported by grants from the Natural Sciences and Engineering Research Council of Canada (NSERC) and a team grant from the Ministry of Education of the Province of Québec (FCAR). S.L. was supported by a graduate research fellowship from NSERC and a Fellowship from the Canadian Federation of University Women.

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LETTERS AND COMMENTS
LETTRES ET COMMENTAIRES

The Stockholm Water Prize and the Experimental Lakes Area

Andy Hamilton's editorial (Can. J. Fish. Aquat. Sci. 48: 1132–1135, July 1991) names many of the original visionaries who were instrumental in forming and initiating the ELA program and some of the administrative and support staff who were instrumental in maintaining the program, particularly in its first decade.

Others were even more instrumental in my receiving the Stockholm Water Prize. Firstly the "mosquito bite brigade" that did the scientific work at ELA. These individuals helped to build and execute projects that we all knew were important and exciting, often ignoring inadequate funding, bad weather, bureaucratic regulations, and civil service time clocks to do so. While a complete list of individuals would be very long and I would undoubtedly miss some, I am especially grateful to the coauthors of one or more of the several papers that were influential in the decision of the Stockholm Water Foundation. These include Ken Beaty, Dana Cruikshank, Ian Davies, Ed DeBruyn, Ev Fee, Dave Findlay, Gary Linsey, Diane Malley, Ken Mills, John Shearer, Mike Stainton, and Mike Turner. All of them shared the good times as well as the bad at ELA for at least 10 years, and in some cases more than 20! In some cases, these individuals passed up opportunities for personal promotion or advancement in order to continue at ELA, in the belief that what they did would make a difference in managing Canada's, and the world's, water resources.

In addition to the above, Suzanne Bayley, Paul Campbell, Ev Fee, Ray Hesslein, Carol Kelly, Bob Newbury, and John Rudd made the design and interpretation of ecosystem-scale experiments fun, improving the scientific quality immensely, and often making it possible to perform an elegant experiment or measurement on an austerity budget by inventing apparatus that we could not afford to purchase. I believe that many of our joint publications were at least as important as those that were cited. Finally, Greg Brunskill, Kaz Patalas, and Gordon Koshinsky provided such thoughtful (and sometimes biting!) reviews of key papers that I could be 100% certain that they would pass peer review of the very best scientific journals. Over the years, Jack Vallentyne, Andy Hamilton, Paul Campbell, Gordon Koshinsky, Ray Hesslein, and John Shearer shouldered much of the administrative burden (that I would've done badly in any case!), making it possible to remain at ELA where the semimonastic environment allowed me to concentrate on science for periods of a duration beyond the belief of most modern ecologists. Donna Laroque and Sharon Ryland translated my indecipherable scrawl into manuscripts and helped organize logistics and communications with Winnipeg for many years.

No scientist has ever had a better group of colleagues, and I thank them all.

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ANNOUNCEMENTS/AVIS

International Symposium on Climate Change and Northern Fish Populations

October 13–16, 1992
Victoria, British Columbia, Canada

First Announcement and Call for Papers

Coordinating Committee

V. Alexander (USA), W. Aron (USA), R. J. Beamish (Canada), J. S. Campbell (Canada), M. Chadwick (Canada), J. C. Davis (Canada), J. Ito (Japan), S. Karnicki (Poland), D. Koshinsky (Canada), P. H. LeBlond (Canada), H. Loeng (Norway), D. A. McCaughran (USA), W. D. McKone (Canada), N. P. Novikov (USSR), S. Pennoyer (USA), H. A. Regier (Canada), B. J. Rothschild (USA), T. Sheng (China), M. Sinclair (Canada), M. P. Sissenwine (USA), J. H. Steele (USA), S. A. Studenetsky (USSR), A. Thompson (Canada), and W. S. Wooster (USA).

Objective

This symposium will promote an exchange of information relating to the effect of climate change on fisheries in aquatic ecosystems in northern latitudes.

Topics

- Evidence for changes in climate and the resulting effects in freshwater and marine environments.
- Effects of climate on fish populations.
- Economic impacts of climate change on fisheries.
- Preparing for climate change.

Publication

Proceedings will be published in the series *Canadian Special Publications of Fisheries and Aquatic Sciences*.

Call for Papers

All interested persons are invited to provide summaries of their papers, not to exceed 250 words, to the Symposium Secretary (address below) by **January 15, 1992.** The Organizing Committee will select papers for oral presentation and for a poster session.

For additional information, please contact the Symposium Secretary, Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, B.C. V9R 5K6, Canada or members of the Coordinating Committee. Telephone: (604) 756-7260; Rapidfax: (604) 756-7053.

Symposium international sur le changement climatique et les populations de poisson nordiques

du 13 au 16 octobre 1992
Victoria (Colombie-Britannique), Canada

Première annonce et demande de communications

Comité de coordination

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Objectif

Le symposium favorisera les échanges d'information sur les effets du changement climatique sur les ressources halieutiques des écosystèmes aquatiques nordiques.

Sujets

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- Effets du climat sur les populations de poisson.
- Incidences économiques des changements climatiques sur les pêches.
- Préparations en vue d'un changement climatique.

Publication

Les comptes rendus seront publiés dans la série *Publications spéciales canadiennes des sciences halieutiques et aquatiques*.

Demande de communications

Les résumés des communications, d'au plus 250 mots, devront être parvenus au Secrétariat du symposium (adresse ci-dessous) au plus tard **le 15 janvier 1992.** Le Comité organisateur choisira des communications pour présentation orale et affichage.

Pour renseignements : Il est possible d'obtenir des précisions en s'adressant au Secrétariat du symposium, ministère des Pêches et des Océans, Station de biologie du Pacifique, Nanaimo (C.-B.) V9R 5K6, Canada, ou en entrant en relation avec les membres du comité de coordination. Téléphone : (604) 756-7260; télécopieur : (604) 756-7053.

Atlantic Salmon Trust Symposium
Pathological Conditions of Wild Salmonids

The Symposium will take place 6–8 May 1992 at the Scottish Office Agriculture and Fisheries Department, The Marine Laboratory, P.O. Box 101, Victoria Road, Torry, Aberdeen, AB9 8DB, Scotland.

For further information, contact Dr. D W. Bruno or Dr. K. MacKenzie at the above address. Telephone: 0224 876544; FAX: 0224 879156.

Symposium du Atlantic Salmon Trust
États pathologiques chez les salmonidés sauvages

Le symposium aura lieu les 6, 7 et 8 mai 1992 au Scottish Office Agriculture and Fisheries Department, The Marine Laboratory, P.O. Box 101, Victoria Road, Torry, Aberdeen, AB9 8DB, Écosse.

Pour tout renseignement complémentaire, s'adresser à D[r] D. W. Bruno ou D[r] K. MacKenzie à l'adresse ci-dessus. Téléphone : 0224 876544; Télécopie : 0224 879156.

Hesslein, R. H., M. J. Capel, D. E. Fox, and K. A. Hallard. Stable isotopes of sulfur, carbon, and nitrogen as indicators of trophic level and fish migration in the lower Mackenzie River basin, Canada **2258–2265**
Dawe, E. G., D. M. Taylor, J. M. Hoenig, W. G. Warren, G. P. Ennis, R. G. Hooper, W. E. Donaldson, A. J. Paul, and J. M. Paul. A critical look at the idea of terminal molt in male snow crab (*Chionoecetes opilio*) **2266–2275**
Sullivan, B. K., P. H. Doering, C. A. Oviatt, A. A. Keller, and J. B. Frithsen. Interactions with the benthos alter pelagic food web structure in coastal waters **2276–2284**
Lalonde, S., and J. A. Downing. Epiphyton biomass is related to lake trophic status, depth, and macrophyte architecture **2285–2291**

Letters and Comments/Lettres et commentaires

Schindler, D. W. The Stockholm Water Prize and the Experimental Lakes Area **2292**

Announcements/Avis **2293–2294**

Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

Volume 48, No. 11, November 1991

Volume 48, nº 11, novembre 1991

Roby, D., J. D. Lambert, and J. M. Sévigny. Morphometric and electrophoretic approaches to discrimination of capelin (*Mallotus villosus*) populations in the Estuary and Gulf of St. Lawrence **2040–2050**

MacIsaac, H. J., W. G. Sprules, and J. H. Leach. Ingestion of small-bodied zooplankton by zebra mussels (*Dreissena polymorpha*): can cannibalism on larvae influence population dynamics? **2051–2060**

Mundie, J. H., K. S. Simpson, and C. J. Perrin. Responses of stream periphyton and benthic insects to increases in dissolved inorganic phosphorus in a mesocosm **2061–2072**

Campbell, A., D. J. Noakes, and R. W. Elner. Temperature and lobster, *Homarus americanus*, yield relationships **2073–2082**

Morgan, J. D., and G. K. Iwama. Effects of salinity on growth, metabolism, and ion regulation in juvenile rainbow and steelhead trout (*Oncorhynchus mykiss*) and fall chinook salmon (*Oncorhynchus tshawytscha*) **2083–2094**

Gordoa, A., and C. M. Duarte. Size-dependent spatial distribution of hake (*Merluccius capensis* and *Merluccius paradoxus*) in Namibian waters **2095–2099**

Stefansson, S. O., B. Th. Björnsson, T. Hansen, C. Haux, G. L. Taranger, and R. L. Saunders. Growth, parr–smolt transformation, and changes in growth hormone of Atlantic salmon (*Salmo salar*) reared under different photoperiods **2100–2108**

Cerrato, R. M. Analysis of nonlinearity effects in expected-value parameterizations of the von Bertalanffy equation **2109–2117**

Tanasichuk, R. W., D. M. Ware, W. Shaw, and G. A. McFarlane. Variations in diet, daily ration, and feeding periodicity of Pacific hake (*Merluccius productus*) and spiny dogfish (*Squalus acanthias*) off the lower west coast of Vancouver Island **2118–2128**

Pelletier, D., and P. Gros. Assessing the impact of sampling error on model-based management advice: comparison of equilibrium yield per recruit variance estimators **2129–2139**

Edge, T. A., D. E. McAllister, and S. U. Qadri. Meristic and morphometric variation between the endangered Acadian whitefish, *Coregonus huntsmani*, and the lake whitefish, *Coregonus clupeaformis*, in the Canadian Maritime Provinces and the State of Maine, USA **2140–2151**

Liskauskas, A. P., and M. M. Ferguson. Genetic variation and fitness: a test in a naturalized population of brook trout (*Salvelinus fontinalis*) **2152–2162**

Skilbrei, O. T. Importance of threshold length and photoperiod for the development of bimodal length–frequency distribution in Atlantic salmon (*Salmo salar*) **2163–2172**

Millar, R. B. Selecting loci for genetic stock identification using maximum likelihood, and the connection with curvature methods **2173–2179**

Lydersen, C., L. A. Angantyr, Ø. Wiig, and T. Øritsland. Feeding habits of Northeast Atlantic harp seals (*Phoca groenlandica*) along the summer ice edge of the Barents Sea **2180–2183**

Matthews, G. B., R. A. Matthews, and B. Hachmöller. Mathematical analysis of temporal and spatial trends in the benthic macroinvertebrate communities of a small stream **2184–2190**

Haas, G. R., and J. D. McPhail. Systematics and distributions of Dolly Varden (*Salvelinus malma*) and bull trout (*Salvelinus confluentus*) in North America **2191–2211**

Doyon, J.-F., C. Hudon, R. Morin et F. G. Whoriskey Jr. Bénéfices à court terme des mouvements anadromes saisonniers pour une population d'omble de fontaine (*Salvelinus fontinalis*) du Nouveau Québec **2212–2222**

Peterson, R. H., J. Power, and D. J. Martin-Robichaud. Morphological basis of the pectoral fin flutter of embryonic Atlantic salmon (*Salmo salar*) **2223–2227**

Paine, M. D., W. C. Leggett, J. K. McRuer, and K. T. Frank. Effects of incubation in oiled sediment on emergence of capelin (*Mallotus villosus*) larvae **2228–2239**

Tyler, A. V., and W. R. Crawford. Modeling of recruitment patterns in Pacific cod (*Gadus macrocephalus*) in Hecate Strait, British Columbia **2240–2249**

O'Gorman, R., E. L. Mills, and J. S. DeGisi. Use of zooplankton to assess the movement and distribution of alewife (*Alosa pseudoharengus*) in south-central Lake Ontario in spring **2250–2257**

(Continued inside back)

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CJFSDX48(11) 2039–2294 (1991)
ISSN 0706-652X

Subscription rates/Abonnements

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